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HERPETIC INFECTION OF THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO*

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To date attempts to infect chicks and chickens with the virus of herpes simplex have been unsuccessful. Recent work in this laboratory has proved the susceptibility of the extra-embryonic membranes of the chick to the viruses of fowl-pox, cow-pox and herpes simplex.[1] This paper is a more detailed account of the experiments dealing with herpes simplex.

Fertile white leghorn eggs are incubated in the usual manner for 12 to 14 days. The eggs are then candled to locate the embryo and air sac. Each of these is ringed with a colored pencil. The surface of the shell overlying the embryo is carefully cleansed with green soap. By means of a cutting carborundum disc attached by a flexible shaft to a small electric motor a square window 1 to 1.5 cm. in diameter is cut in the shell over the embryo. The window is then coated with a thin layer of paraffin and gently stripped from the underlying shell membrane. Exercising diligent aseptic precautions the underlying shell membrane is removed by cutting around the edges of the window with blunt pointed scissors. This exposes the extra-embryonic membranes, the chorion being uppermost. This membrane may then be easily inoculated. By means of a transfusion syringe filled with a mixture of sterile paraffin and vaseline the edges of the window are ringed, a sterile coverslip is placed over the opening and the edges sealed with a hot needle.

The herpetic virus used in this work was the HF strain obtained from the Rockefeller Institute. Glycerinated brain was emulsified

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and inoculated intracerebrally into a rabbit which was killed 4 days after developing a typical herpetic encephalitis. The brain was removed aseptically and preserved fresh in the ice-box, as well as in glycerin. Small fragments of fresh brain (approximately 1 mm. cubes) were used for inoculation. Transplants were made after 48 hours by removing the membrane sterilly and introducing fragments of the infected membrane into other eggs. It was found that this method was rather tedious, consequently an attempt was made to inoculate eggs by using ground, emulsified brain and to transplant by inoculating ground, emulsified membranes, with the result that more abundant infections developed. The virus has been carried through six generations in the chick membranes.

In experiments using tissue fragments for inoculation changes may be noted in the chorio-allantoic membranes within 12 hours. The first change is a congestion of the vessels. Within 24 hours one sees around the bit of inoculum a rather opaque, grayish red zone which spreads peripherally. Careful examination at this time reveals within and around this zone minute grayish opacities just visible at a magnification of 30 diameters. These are particularly abundant along the course of the blood vessels. At 36 hours well defined, raised, grayish opacities, some of which are more than 1 mm. in diameter, are visible to the naked eye. As the lesion progresses these smaller areas tend to coalesce so that at the end of 72 to 96 hours the whole of the exposed membrane may be involved. Inoculation of ground, emulsified material leads to similar lesions. This method, however, provokes a more diffuse reaction which develops more rapidly. By either method the lesions are focal in distribution.

In this work some of the embryos die, due presumably to trauma. A large percentage of those that survived the inoculation were sacrificed at 2, 3, or 4 days for histological study and for further transfers. A few were allowed to run their course undisturbed. In some the process involved the membranes extensively and led to the death of the embryo; in others the involvement was less extensive and the chick hatched in the usual manner. Such chicks showed no gross changes. Examination of embryos which died also showed no changes. At other times the lesions developed in the usual manner and then regressed, ending in complete gross resolution. One egg which showed typical gross lesions with subsequent complete resolution was reinoculated, resulting in another characteristic "take."

For microscopic study the membranes were fixed in Zenker's solution and stained with hematoxylin and eosin or with eosin and methylene blue. The vessels throughout the entire membrane appear congested and there is some hemorrhage. There is a marked polymorphonuclear leukocytic reaction beneath the ectoderm of the chorion. In addition one sees large mononuclear leukocytes scattered here and there. In older lesions, particularly those in which tissue fragments served as the inoculum, there are many fibroblasts. In one instance there is a marked hyperplasia of ectoderm with a minimal amount of necrosis. In another, necrosis is the important feature and there is little or no hyperplasia. In any event some of the cells which remain show the characteristic intranuclear changes of infection due to the virus of herpes simplex.

Within the nuclei of ectodermal cells one sees inclusions of different size, homogeneity and staining reaction. This type of change is usually seen in groups of cells which correspond to the focal lesions noted in the gross, but it may be observed in isolated cells. The nucleus is almost invariably swollen and the chromatic material is collected along the nuclear membrane. Within such cells one sees usually one, but sometimes two or more intranuclear masses distinct from the nucleoli. Some of these bodies are quite small, no larger than nucleoli, others are so large that they almost completely fill the greatly swollen nuclei. They are separated from the nuclear membrane by a clear zone, and are acidophilic in reaction. The smaller bodies appear to be composed of minute granules, while the larger ones are more homogeneous and hyaline in appearance. The largest inclusions stain basophilically and under high magnification appear to be composed of closely packed, minute, basophilic granules. Within other nuclei the inclusion may completely fill the nuclear space. These inclusions stain faintly and appear very finely granular. Such nuclei may become exceedingly large, sometimes attaining a volume considerably greater than that of the entire normal cell. In these nuclei there is a delicate bluish reticulum which appears to divide the nucleus into many small compartments. These compartments are filled with minute, almost invisible, lavender granules. The cytoplasm of these cells shows few if any changes. Occasionally it appears foamy, but this is not the rule.

Discussion

It is of considerable interest that one is able to infect the chorio-allantoic membranes of white leghorn chick embryos with a strain of herpes simplex virus which is innocuous to adult white leghorn chickens both by corneal and by cutaneous inoculation. The gross lesions of the herpetic membranes are entirely comparable to those of the herpetic keratitis of rabbits. Specific intranuclear inclusions are seen in the cells of the infected membranes. Both eosinophilic and basophilic inclusions are present and are characteristic of the herpetic inclusions observed in other experimental animals. The peculiar reticulum and minute lavender granules within many of the swollen nuclei constitute a very interesting change somewhat suggestive of that described by Goodpasture and King,[2] and again by Goodpasture and Woodruff,[3] in the cytoplasm of epithelial cells within the lesions of molluscum contagiosum. It has not been possible, however, to demonstrate any "elementary corpuscles" similar to those described by Borrel in fowl-pox, by Lipschütz in molluscum contagiosum, and by Paschen in vaccinia.

It has been suggested by Goodpasture and his coworkers[1] that the method of inoculating chick embryos may be of value in the study of various other virus diseases, including those which have not been engrafted upon a foreign host. The fact that the embryonic membranes of the chick are more susceptible than baby or adult chicks points to such a conclusion.

Conclusions

1. The chorio-allantoic membranes of the chick embryo are susceptible to infection with a strain of herpes simplex virus which is innocuous to adult chickens of the same breed.

2. The microscopic lesions of these membranes are like those of herpetic lesions of mammals.

3. A peculiar nuclear change in ectodermal cells is described, characterized by enormous enlargement of the nucleus, and by a partitioning of it by delicate trabeculae into compartments which are filled by minute, uniform and faintly stained basophilic granules.

REFERENCES

1. Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J. The cultivation of vaccine and other viruses in the chorio-allantoic membrane of chick embryos. *Science*, 1931, **74**, 371.

2. Goodpasture, E. W., and King, Howard. A cytologic study of molluscum contagiosum. *Am. J. Path.*, 1927, **3**, 385.

3. Goodpasture, E. W., and Woodruff, C. Eugene. A comparison of the inclusion bodies of fowl-pox and molluscum contagiosum. *Am. J. Path.*, 1931, **7**, 1.

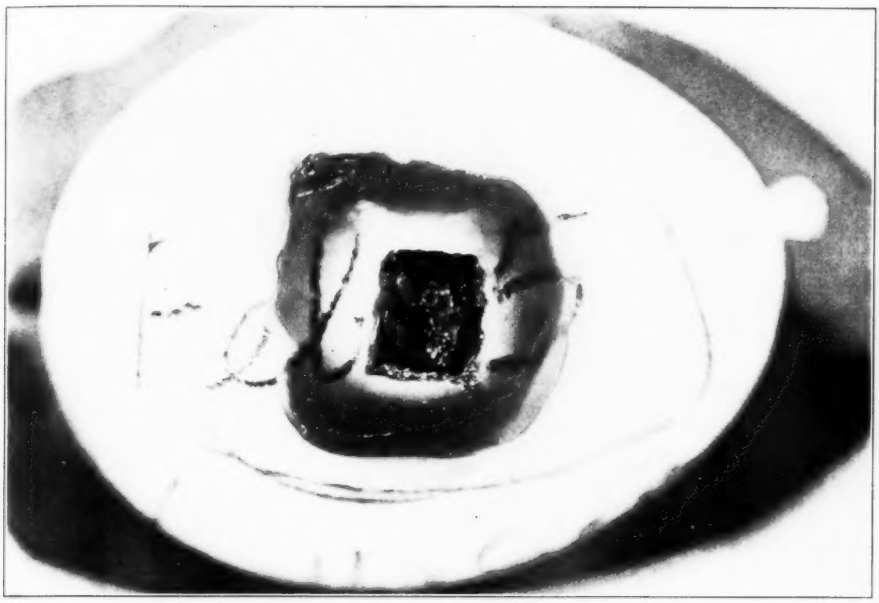
DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Gross photograph showing the glistening pox on the chorio-allantoic membrane of a 48 hour inoculated egg. × 1.5.

FIG. 2. Photograph of portion of above membrane magnified eight times.

FIG. 3. Photomicrograph of 36 hour membrane showing the diffuse, small, pox-like eruptions. × 10.



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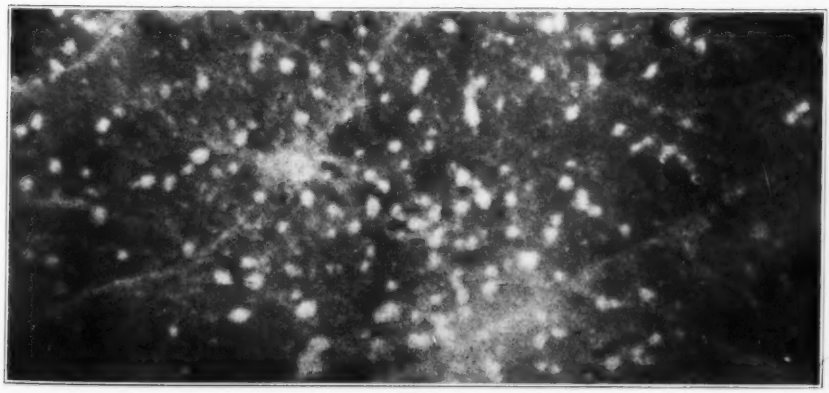

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Dawson Herpetic Infection of Chorio-Allantoic Membrane

Plate 2

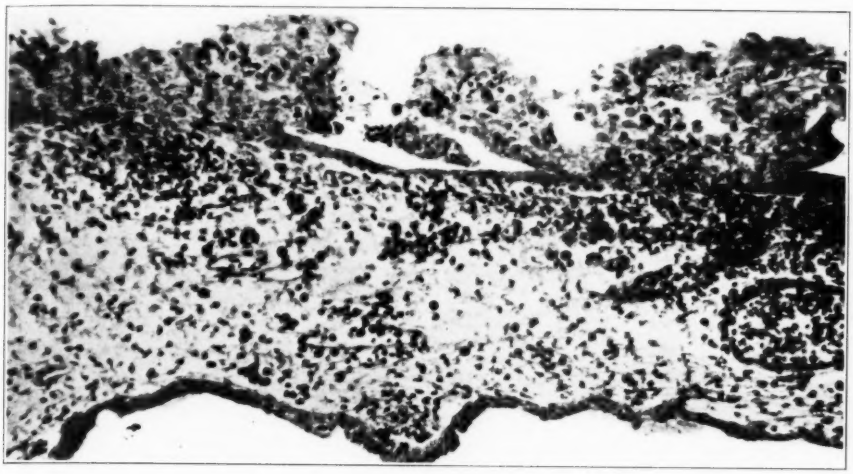
Fig. 4. Photomicrograph of entire chorio-allantoic membrane at the site of a hyperplastic lesion. Inclusion bodies may be seen within the nuclei of epithelial cells. The inflammatory exudate beneath the lesion is also illustrated. Eosin-methylene blue stain. × 200.

Fig. 5. Ectoderm of chorion showing several large, very finely granular inclusions which completely fill the nuclei. Eosin-methylene blue. × 1800.

Fig. 6. Ectoderm of chorion showing several smaller intranuclear inclusions which appear granular. Eosin-methylene blue. × 1800.

Fig. 7. Two large ectodermal cells from the hyperplastic chorion. The one on the right shows a poorly defined cytoplasm represented by the clear zone around the greatly enlarged nucleus. The dark staining reticulum is illustrated. The finely granular material can be seen within the compartments formed by the interlacing trabeculae. The cell on the right shows the finely granular appearance of the large inclusions without the reticular material. Eosin-methylene blue. × 2300.

Fig. 8. Two intranuclear inclusions in the ectodermal cells of the chorion, similar to those in Fig. 2. Eosin-methylene blue. × 1800.



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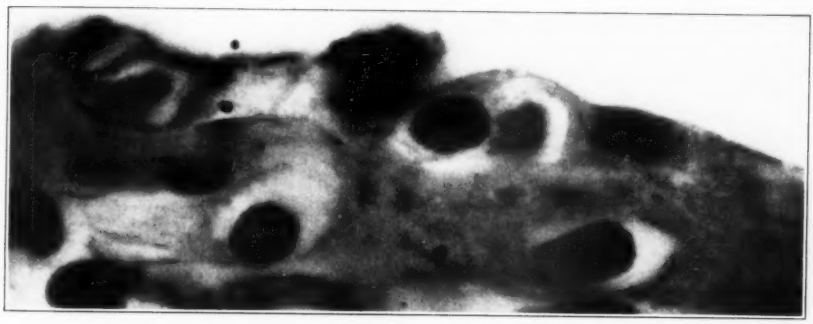

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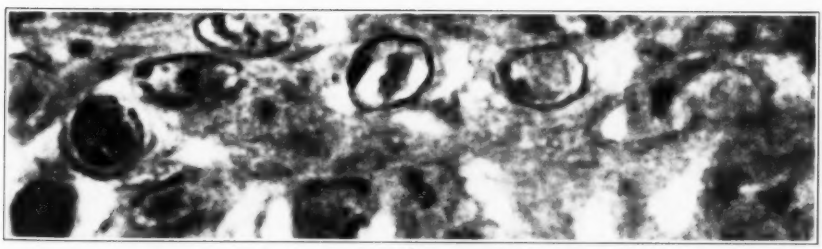

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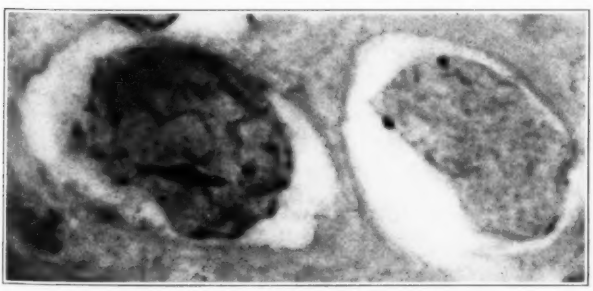

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Dawson Herpetic Infection of Chorio-Allantoic Membrane

CELLULAR INCLUSIONS IN CEREBRAL LESIONS OF LETHARGIC ENCEPHALITIS *

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In many of the articles dealing with encephalitis lethargica the previous work on the subject has been reviewed. Consequently no attempt will be made here to discuss the literature. Excellent summaries may be found in the papers of Zinsser,[1] von Economo,[2] and in the report of the Matheson Commission.[3]

Many investigators have implicated a filterable virus as the etiological factor in encephalitis lethargica, and some have claimed success in transmitting an encephalitis to experimental animals by the inoculation of brain or nasopharyngeal washings. In every instance, except one in which an encephalitis has been transmitted in series in experimental animals, the virus has been proved identical with that of herpes simplex. In the one exception the virus was that of rabies. Only nine strains of herpetic virus have been isolated from human encephalitis.[3] It has been the experience of most workers that repeated inoculations of material from encephalitic patients have uniformly led to negative results. Furthermore, Flexner and Amoss [4] have isolated a strain of herpes virus from the spinal fluid of a luetic patient in whom they found evidence neither of herpes nor of encephalitis. From the numerous negative results which have been published, and the large number which undoubtedly have not been published, it must be concluded that the isolation of a virus from cases of lethargic encephalitis infectious for laboratory animals is, at least, an unusual occurrence. The association of herpetic virus with human encephalitis seems, on the basis of available evidence, adventitious.

At the present time, diseases having filterable viruses as etiological agents are for the most part characterized by certain cytological changes in their specific lesions. These may be in the form of inclusion bodies within the nucleus or the cytoplasm, hyperplasia of cells

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or simply necrosis of individual cells. In spite of the fact that herpes simplex is characterized by definite intranuclear inclusion bodies, the workers who have implicated this virus in the etiology of human encephalitis have never demonstrated in human cases inclusions characteristic of herpetic infections. Herzog,[5] Da Fano,[6] and others have described vague intracellular bodies which are not at all typical of virus inclusions.

It is the purpose of this paper to present a case of human encephalitis, clinically encephalitis lethargica, in which intranuclear inclusion bodies were found. These inclusions are quite definite, and while they simulate in certain respects the inclusions associated with herpetic infections, there are certain morphological differences. Furthermore inoculations of brain tissue upon the cornea and intracerebrally in rabbits failed in every case to induce herpetic infection.

Report of Case

Clinical History: The patient, W. W., white, age 16 years, was brought to the Out-Patient Department July 18, 1931. His mother, who brought him to the clinic and gave the history, said that he had had a sunstroke. Additional points in the history were obtained by a social service worker.

Sixteen months ago the patient was found on the street in an unconscious condition and was taken home. No other details could be obtained. Twelve months ago, while attending a boys' camp, he is said to have had a sunstroke. He was taken to a hospital which recorded the presence of headache, sore throat, a diffuse rash over the body, temperature of 99° F, and clear spinal fluid under normal pressure with 14 cells. He recovered from this attack rapidly.

Eight months ago he had a light attack of influenza, but did not go to bed and had no doctor. Two weeks ago while working in the sun with his father he suddenly said, "I feel just like cussing you." A few nights later his sister came home and said that the patient was in a park, acting queerly and staring at trees. About this time he started walking about at night, refused to undress, was quite nervous and smoked a great deal. One week before admission he began having involuntary jerking movements of the arms and legs. Other information obtained without definite dates of onset was that he had gradually become very slow and deliberate in his movements and his memory had failed considerably. He was referred to as "the boy who slept standing up" by one of his recent employers. For some time he had had diplopia.

On admission to the Vanderbilt Hospital his temperature was 99° F, pulse 32, respirations 20. He was well developed and nourished. Voluntary movements and speech were slow and deliberate. The face had a typical mask appearance. During the examination he got out of bed several times and walked around the room. In walking the head was bent slightly forward. Occasionally there was an involuntary, convulsive jerking of the arms and legs. The head presented nothing of interest. The pupils were equal, regular and round. They reacted normally. The arms offered some rigidity to passive motion. The superficial

and deep reflexes were equal and hyperactive. There were no pathological reflexes. The neck was moderately stiff. There was a slight, fine tremor of the fingers. One observer noted the presence of "herpes" on July 29, eleven days after admission. How long these lesions had been present was not stated.

Laboratory Findings on Admission: Blood: White blood cells 6200, 73 per cent polymorphonuclear leukocytes, 24 per cent lymphocytes. Hemoglobin 14.5 gm. Wassermann negative.

Urine: Normal.

Spinal Fluid: Slightly increased pressure, clear, 14 cells, sugar 58 mg., globulin positive. Wassermann negative.

Course in Hospital: While in the hospital little was done for the patient with the exception of sedatives and forced feedings. During the first week the temperature chart showed variations from 98.6 to 100.8° F, usually reaching its peak at 4.00 or 8.00 P.M. Toward the end of the second week it reached 101.8° F. For two weeks it ranged from 90.6 to 102°; following this it showed fairly marked fluctuation with a gradual tendency to rise higher each day until he died on August 31. At this time his temperature was 106° F. His course was progressively down hill. He became more and more lethargic, and the convulsions and twitchings increased in severity and frequency. His expression became typically masked and he exhibited a definite lead-pipe rigidity of the extremities. Toward the end of his illness the convulsive twitchings became almost constant. Repeated examination of the spinal fluid showed little change except for a slight decrease of sugar and number of cells. Guinea pig inoculations and smears of spinal fluid were repeatedly negative.

Pathological Anatomy: Grossly, the brain showed no changes of a specific nature. The meninges were smooth, of normal thickness and glistening. There was no demonstrable increase in the amount of cerebrospinal fluid, nor was this fluid cloudy or blood-tinged. The vessels within the subarachnoidal space were definitely congested. Transverse sections through the brain showed a diffuse congestion and some edema. There were no definite areas of hemorrhage or necrosis.

Microscopic Examination

Microscopically, numerous changes are seen. These changes fall into two definite groups, the one affecting the blood vessels, the other individual cells. The sections studied were taken from all of the important cerebral structures, the spinal cord and the cerebellum.

The lesions associated with the blood vessels are congestion, hemorrhage and a lymphocytic infiltration of the adventitial coat of the smaller arteries and veins. These changes are seen in all the sections studied, with the exception of those taken from the cerebellum and spinal cord. In spite of their wide distribution these

changes are most evident in the sections from the cortex, the lenticular, caudate and red nuclei, and the thalamus. Examples of these changes are seen in the white as well as the gray matter. The congestion is the most constant finding, minute areas of hemorrhage and perivascular infiltration being somewhat less common. The areas of hemorrhage are always quite small and are seen around the capillaries chiefly. The cellular infiltration is composed almost entirely of lymphocytes. An occasional plasma cell is seen, but there are no polymorphonuclear leukocytes. While this exudate is rather scanty it is nevertheless quite definite. It is not a perivascular infiltration in the sense that the cells lie within the perivascular space. Close examination shows them to be enmeshed by the fibers of the vessel wall, particularly the adventitial sheaths. The smaller arteries are the vessels chiefly affected. No thrombi are seen within any of the blood vessels.

The changes affecting the cells are of four types. In eosin-methylene blue preparations they may appear in the form of eosinophilic intranuclear bodies entirely separate from the nucleolus of the cell, with or without irregular eosinophilic cytoplasmic bodies, or as simple degeneration and necrosis, or as phagocytosis of such cells. These changes are found in all parts of the cerebrum, but are most obvious in the left motor cortex, the red nucleus and the caudate nucleus. The intranuclear bodies may appear without any accompanying cytoplasmic bodies or any detectable degenerative changes. The intracytoplasmic masses are not seen in cells which do not show the intranuclear bodies and are almost invariably accompanied by degenerative changes of various types within the cell. Other cells may undergo degenerative changes without exhibiting bodies within the nucleus or the cytoplasm.

The intranuclear masses occur chiefly in the large ganglion cells, but are also seen rarely in neuroglial cells. There may be one or several within the nucleus, usually only one. They vary in size from those which are perhaps twice as large as the nucleolus to some which completely fill the nucleus. They may be round, oval or horseshoe-shaped. Associated with this variation in size there is a corresponding variation in appearance. The smaller bodies tend to be more eosinophilic and present a more granular appearance. The larger bodies are more homogeneous and in most instances tend to have a lavender color. In affected cells the chromatic material of the nu-

cleus is concentrated along the nuclear membrane. These intranuclear bodies are entirely separate and easily distinguishable from the nucleoli.

The cytoplasmic bodies occur only in ganglion cells. They are less definite in their appearance, are usually multiple, and vary in size from very small rounded masses to those which almost fill the cytoplasm. They are correspondingly irregular in shape. Their staining reaction is much more uniform. They appear as homogeneous, hyaline, pink-staining masses. As to distinctness of outline they again vary considerably. Many are rather sharply defined, but equally as many merge almost imperceptibly with the cytoplasm of the cells. In only a few instances are they surrounded by a halo.

The degenerative changes are frequently associated with the bodies just described. In many instances, however, one sees degenerating cells which are not so involved. Surrounding such cells one frequently sees accumulations of apparently healthy cells which vary in shape from round to spindle. These cells have rather scanty cytoplasm which stains lavender. Their nuclei are large as compared to the cytoplasm. Occasionally, one sees a necrotic ganglion cell which has been invaded by one of these cells. Such lesions are interpreted as pseudo- and true neuronophagia respectively. The nature of the phagocytic cells, whether neuroglial or microglial in origin, is unknown.

What appears as a later stage of the process of phagocytosis is the accumulation of cells, resembling the phagocytic cells, which are occasionally seen unassociated with degenerating or necrotic nerve cells. These phagocytic cells appear as rosettes. In some instances one is able to see small purplish fragments of material which resemble calcium in the center of such a rosette. These changes are seen most commonly in the pons, the lenticular and the caudate nuclei.

In addition to the changes found in the nervous system there was some bronchopneumonia, bilateral fibrinous pleuritis, and a few petechiae within the mucosa of the gastro-intestinal tract.

Discussion

The inclusion bodies found within the nerve cells and neuroglial cells are comparable morphologically to those seen in the lesions of herpes simplex, zona, varicella and virus III disease of rabbits. Of

these four types they resemble the inclusions seen in herpes simplex most closely. There are, however, several differences. The inclusions seen in this case never appear as extremely large, finely granular, lavender bodies which completely fill the nucleus. In herpetic lesions the intracytoplasmic masses are not seen. The inclusions in this case are more numerous in the cortex and are accompanied by a minimal amount of necrosis, while in herpetic encephalitis of rabbits the brain stem and mesencephalon are chiefly involved and there is widespread necrosis of nerve cells. One is able to conclude quite definitely that the cellular inclusions are not associated with the virus of herpes simplex because of the negative results of intracerebral inoculations of emulsified brain into rabbits.*

In view of the present state of knowledge as to the relation which exists between inclusion bodies and filterable viruses, it is suggested that in this particular case a filterable virus may have been associated with the lesions. That this is a case of encephalitis there can be no doubt. From the history, physical examination and laboratory findings, it fits quite well into the amyostaticakinetic form of encephalitis lethargica, the terminal attack being an acute exacerbation of a preëxistent infection of sixteen months duration.

A second case has recently come to us which presented a typical clinical picture of an acute lethargic encephalitis of only forty-eight hours duration. Postmortem examination of the brain revealed gross congestion, edema, microscopic hemorrhages and capillary thrombosis, unassociated with perivascular or epivascular lymphocytic infiltration. Careful examination of the nerve cells revealed extensive necrosis and some pseudoneuronophagia. No inclusions were observed.

Another case examined at autopsy was a typical clinical encephalitis lethargica coming on after extensive burns over the body, several weeks elapsing between the burns and the development of the symptoms referable to the central nervous system. Grossly, little could be demonstrated other than congestion of the basal ganglia. Microscopically, there were numerous lymphocytes within the arterial walls, extensive necrosis, a diffuse mononuclear exudate, and

* The material used for animal inoculation consisted of small fragments of basal ganglia which had been preserved in 50 per cent glycerine-saline solution and kept at freezing temperature for 1 month and 16 days. This material was washed, ground and emulsified in saline and inoculated intracerebrally into 2 monkeys (*M. rhesus*), 9 mice, 1 guinea pig, and 17 adult rabbits. One rabbit was inoculated in the testis.

little hemorrhage. The lesions were almost entirely confined to the basal ganglia. Here again no inclusions were demonstrated, and animal inoculations were negative.

These three cases may well be included in the group of encephalitis lethargica. Each is distinctly different from the other clinically, as well as pathologically. In fact, they present only a few points of similarity. In only one of them have inclusion bodies been demonstrated. In this particular case there is strong morphological evidence which points to a filterable virus as the etiological agent. Careful study of the other two cases revealed no inclusions, and it is difficult to believe that other investigators could have overlooked them in cases previously reported. It must be concluded that the case here described differs from others heretofore recorded in this essential feature.

If, then, in this small series there is evidence pointing to at least one cytological difference between cases which are called at present epidemic or lethargic encephalitis, it may be concluded that encephalitis lethargica is probably not a clinical or pathological entity, but that it represents an ill-defined group characterized by varied clinical and pathological findings and probably caused by various agents.

Experimentally, considerable work was done with material from all three of our cases, using fresh and glycerinated tissue from the basal ganglia, cortex and medulla as inocula. Material from the three cases was washed, ground, emulsified in saline and inoculated into a total of four baby rabbits, thirty-one adult rabbits, baby mice, adult mice, two puppies, three monkeys and several guinea pigs and cats. In most instances the emulsified brain was inoculated intracerebrally, although several attempts were made to infect the rabbit's cornea. In rabbits, monkeys and guinea pigs, 0.5 to 1 cc. of a thick emulsion was inoculated. Following Flexner's work on poliomyelitis attempts were made to infect animals by following the intracerebral inoculation with intraperitoneal injections of emulsified brain. The animals were watched for varying periods. Examination of their brains revealed not the slightest suggestion of any inflammatory process comparable to that seen in encephalitis.

Many of the animals survived without manifesting any clinical changes. These were observed over periods varying from three to six months. Careful temperature charts were kept on all animals.

In none of these animals was there any significant fever. Microscopic examination of the brains of animals which survived several weeks and were sacrificed showed no significant changes.

In another case, clinically diagnosed encephalitis lethargica (brother to Case 2), spinal fluid was inoculated intracerebrally into rabbits in 0.5 and 1 cc. quantities with negative results. This child survived the acute attack. At the present time, six months after onset, the child shows a reversal of the sleep cycle and some salivation, which is interpreted by the clinicians as evidence of encephalitic sequellae.

Summary and Conclusions

1. A case of lethargic encephalitis is reported in which intranuclear and intracytoplasmic "inclusions" occur.

2. It is suggested, on the basis of the presence of cellular inclusions, that this case of encephalitis may have been due to a cytotropic virus.

3. Two other cases of encephalitis are mentioned in which no inclusions were found.

4. It is further judged from an etiological standpoint that lethargic encephalitis may not be a distinct entity.

5. Animal inoculations with material from each case induced no demonstrable infection.

REFERENCES

1. Zinsser, H. The present state of knowledge regarding epidemic encephalitis. *Arch. Path.*, 1928, **6**, 271.

2. von Economo, C. Encephalitis Lethargica, its Sequellae and Treatment. Oxford University Press, London, 1931.

3. Epidemic Encephalitis. Report of a survey by the Matheson Commission. Columbia University Press, New York, 1929.

4. Flexner, S., and Amoss, H. L. Contributions to the pathology of experimental virus encephalitis. An exotic strain of encephalitogenic virus. *J. Exper. Med.*, 1925, **41**, 215.

5. Herzog. Zur pathologischen Anatomie der sog. Encephalitis epidemica. *München med. Wchnschr.*, 1921, **68**, 191.

6. Da Fano, D. The histo-pathology of epidemic (lethargic) encephalitis. *Brit. M. J.*, 1921, **1**, 153.

DESCRIPTION OF PLATES

PLATE 3

FIG. 1. Longitudinal section through small venule in the lenticular nucleus. The lymphocytes are seen in the adventitial coat. Hematoxylin and eosin stain. × 100.

FIG. 2. Large ganglion cell from left motor cortex showing several dense cytoplasmic inclusions surrounded by halos, and a single, poorly defined intranuclear inclusion. Phagocytic cells are beginning to accumulate around it. Eosin-methylene blue stain. × 1100.

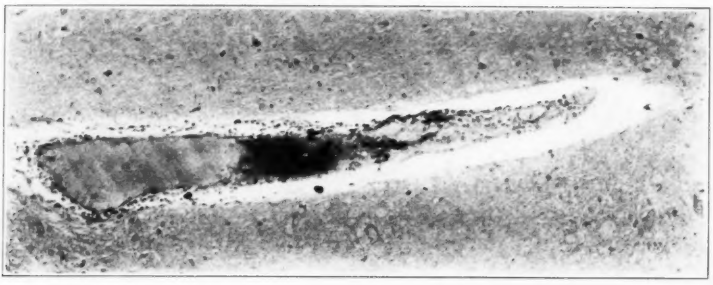
FIG. 3. Pseudoneuronophagia of a ganglion cell from the caudate nucleus. The cell contains a definite intranuclear inclusion and a single pale, rounded cytoplasmic inclusion near the upper pole of the nucleus. Eosin-methylene blue. × 1100.

FIG. 4. Ganglion cell from left motor cortex showing the characteristic large granular inclusions and three poorly defined, homogeneous, cytoplasmic inclusions. This cell shows the characteristic condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. × 2500.

FIG. 5. Necrotic ganglion cell from pons surrounded by phagocytic cells. Eosin-methylene blue. × 1100.

FIG. 6. Ganglion cell from left motor cortex showing a granular intranuclear inclusion and a large homogeneous cytoplasmic inclusion. Eosin-methylene blue. × 2500.

FIG. 7. Two swollen neuroglial cells from caudate nucleus showing small intranuclear inclusion. Eosin-methylene blue. × 1100.



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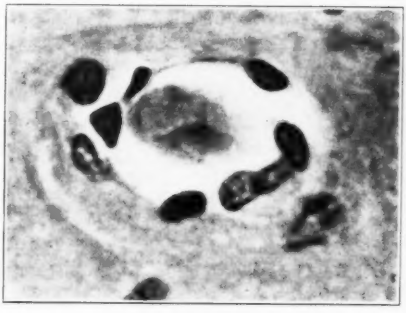

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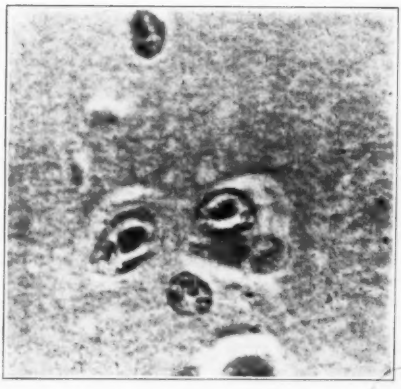

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Dawson

Cellular Inclusions in Lethargic Encephalitis

PLATE 4

FIG. 8. Ganglion cell from caudate nucleus showing an eosinophilic, granular, intranuclear inclusion and a poorly defined, round, homogeneous cytoplasmic inclusion. Surrounding the cell are many phagocytes. Eosin-methylene blue. × 2500. (See Fig. 3 for photomicrograph of this cell.)

FIG. 9. Ganglion cell from left motor cortex showing large, granular, eosinophilic intranuclear inclusion and three indefinite homogeneous cytoplasmic inclusions. The chromatic material is collected along the nuclear membrane. Eosin-methylene blue. × 2500. (See Fig. 4 for photomicrograph of this cell.)

FIG. 10. Ganglion cell from left motor cortex showing characteristic intranuclear inclusion. Eosin-methylene blue. × 2500.

FIG. 11. Ganglion cell from left motor cortex showing several reddish orange, homogeneous, cytoplasmic inclusion bodies which are surrounded by halos. There is also a large granular intranuclear inclusion. Eosin-methylene blue. × 2500. (See Fig. 2 for photomicrograph of a similar cell.)

FIG. 12. Ganglion cell from left motor cortex showing a single intranuclear inclusion. This cell also illustrates the condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. × 2500.

FIG. 13. Ganglion cell from caudate nucleus showing a typical intranuclear inclusion. Eosin-methylene blue. × 2500.

FIG. 14. Ganglion cell from caudate nucleus showing an intranuclear inclusion with some condensation of the chromatic material along the nuclear membrane. Eosin-methylene blue. × 2500.

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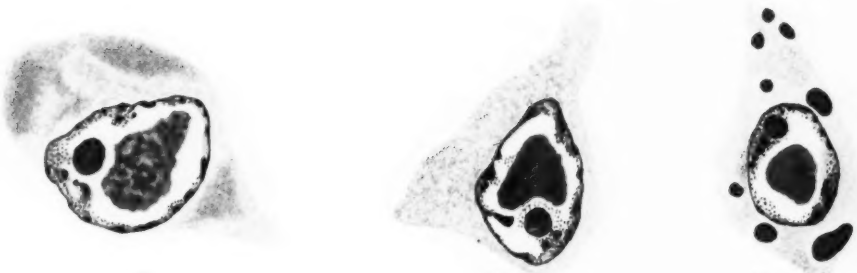

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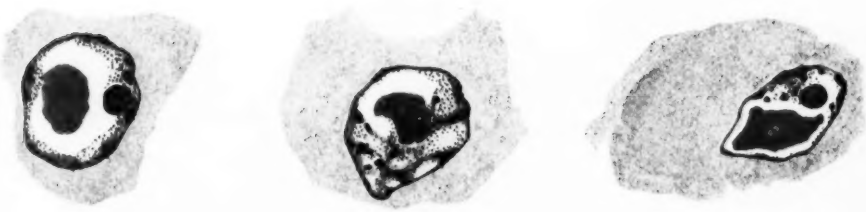

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TUBERCULOUS VEGETATIONS OF THE TRUNK OF THE PULMONARY ARTERY *

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Although nearly fifty cases of tuberculous involvement of the aorta have been reported, tuberculosis of the main stem of the pulmonary artery is rare. Only two cases are described in the literature and these deal with miliary lesions. The first case, reported by Weigert [1] in 1886, represents the extension of a tuberculous lymphadenitis into and through the vessel wall with production of miliary intimal tubercles. Thorel [2] mentioned a second case in which there were very minute miliary intimal tubercles associated with miliary tuberculosis of the main stem of the pulmonary artery. In view of the rarity of the condition the following case is reported.

REPORT OF CASE

Clinical History: L. B., a 65 year old negress, was admitted to the Medical Service of the Cleveland City Hospital complaining of a sore throat. The history of her illness indicated pulmonary disease of at least three weeks duration. The physical examination disclosed a negress of 65 years who appeared severely ill, and who showed evidence of marked loss of weight. The respirations were very rapid and shallow. Over the upper half of the right chest anteriorly, dullness and many fine râles were elicited. The heart sounds were distant and scarcely audible. The rate was rapid. The liver was moderately tender to palpation. The ankles and feet were edematous. The patient died on the day following admission.

POSTMORTEM EXAMINATION

At autopsy, two hours after death, there was observed a generalized miliary tuberculosis. The lungs, in addition, showed tuberculous pneumonia. There was an adherent pericardium. The heart with pericardium weighed 450 gm. Aside from a slight dilatation of the chambers, the heart was normal. At the bifurcation of the pulmonary artery there was a blue-gray, oval, intimal plaque 0.9 cm.

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by 1.5 cm., elevated about 1.5 mm. above the surface. From the middle of this plaque hung a soft, pink-gray polyp 1.5 cm. long and 0.6 cm. thick (Fig. 1). This polyp broke on handling, allowing a thick, pinkish yellow, turbid fluid to escape. A smear of this material showed dense masses of acid-fast bacilli with a little cellular débris. The surface of this polyp was granular and definitely nodular. Below this vegetation and slightly to the left, there was another pedunculated polyp 1 cm. long and extending upward from a point immediately to the left of the commissure joining the right anterior and posterior cusps. The stalk was cylindrical, 3 mm. in diameter and 5 mm. in length. The distal part was ovoid in shape, 5 mm. in diameter and 5 mm. long, and finely granular on the surface. It was gray in color and quite firm, although friable. At the base and to the left of this second vegetation there was a third polyp about 5 mm. in length with a granular surface and bifid upper pole. This polyp had the same color and consistence as the second. The valve leaflets and commissures were normal.

There was a marked tuberculous mediastinitis. Attached to the posterior aspect of the pulmonary artery, near its bifurcation, there were caseous lymph nodes which were also adherent to the tissues at the bifurcation of the trachea. There were numerous other caseous lymph nodes in the vicinity which were matted together by fibrous tissue.

Microscopic Examination

Microscopically the sections show tuberculous granulation tissue between the pulmonary artery and the aorta. While the latter is not affected by the disease, the pulmonary artery throughout its adventitia is severely involved by the tuberculous inflammation. Large areas of caseation necrosis with the usual lymphocytic and endothelial cells are seen. At various levels of the pulmonary artery the media is invaded and interrupted by the tuberculous granulation tissue (Fig. 2). In these situations direct continuity exists between the tuberculous tissue of the adventitia and that of the intima. The intimal disease is characterized by the presence of tuberculous granulation tissue, areas of caseous necrosis, and by medium-sized blood vessels whose walls are markedly thickened. The thickening is eccentric, encroaches upon the lumen and consists of disoriented, loosely arranged hyaline fibrils in which numerous, poorly staining,

large mononucleated cells are enmeshed (Fig. 3). There is also a marked hyaline swelling of the internal elastic lamina of the pulmonary artery to about twice its normal thickness (Fig. 4).

Further continuity exists between the tuberculous tissue of the intima and similar tissue composing the base of the polyp situated at the commissural level. At higher levels this polyp consists of a center of loosely arranged, acellular, hyalinized, fibrinous material and a periphery of more compact hyaline material in which a few lymphocytes are present. A section of the large polyp exhibits a thin marginal ring of compact hyaline material containing an occasional lymphocyte. Dense masses of acid-fast bacilli are present in the center of the ring. One section through the base of the smallest vegetation shows tissue similar to that of the adjacent polyp with which it is continuous. The exact relation of the smallest polyp to the tissues composing the wall of the pulmonary artery was not established.

The continuity of the caseous tuberculous lymphadenitis with the extensive adventitial involvement of the pulmonary artery, and the further continuity, through the interruptions in the media, with the intimal tuberculosis, indicate that the tuberculous arteritis probably began in the adventitia and that the intimal lesions represent extension by contiguity. It is possible that the miliary dissemination had its origin in the tuberculous polypi.

Discussion

In Weigert's [1] case of vascular tuberculosis the disease began as a perivascular lesion which involved the intima only secondarily. Benda,[3] however, reported cases of intimal tuberculosis (not involving the pulmonary artery) in which the disease was confined to the intima. This, he assumed, represented the lesion caused by implantation of tubercle bacilli upon the intact or diseased intimal surface. He claimed that this endangitis tuberculosa represented the more common form of vascular tuberculosis. Subsequent writers and more modern textbooks of pathology are agreed that the periangitic form, first described by Weigert, is by far more common than the endangitic form. Jores [4] further emphasized the fact that tuberculous vascular involvement is more common with veins than with arteries.

The appearance of the intimal tubercle varies from a small,

slightly elevated nodule, miliary in size, to soft, pedunculated polypi 1 cm. in length, and larger. Thus Benda described a "very large" polyp of the main branch of the pulmonary vein, which hung almost into the left ventricle. The polypi are usually yellow or yellow-gray in color. The external surface has been described as smooth and also as nodular. The consistence is usually quite soft, although an occasional polyp of firm consistence is seen.

The contents of the soft polypi consist of caseous, pus-like material in which a varying number of acid-fast bacilli are found. Usually the bacillary content is very high. Benda called attention to the fact that these polypi are often ulcerated, even though the surface appears smooth and uninterrupted. A layer of hyalinized fibrin may cover the point of rupture and give it the appearance of a smooth surface. Such a lesion may then be the source of a disseminated miliary tuberculosis.

Although no polypi of the pulmonary artery have been described, the description of polypi found in the aorta and pulmonary veins as given above adequately covers the vegetations found in the pulmonary artery of this case.

Summary

1. A third case of tuberculous involvement of the main stem of the pulmonary artery has been described.

2. The tuberculous involvement in this case is the result of an extension of a tuberculous lymphadenitis from adjacent lymph nodes to the adventitia and thence to the intima of the pulmonary artery.

I am indebted to Prof. Howard T. Karsner for helpful criticism and suggestions in the preparation of this paper and to Dr. Alan R. Moritz for the photomicrographs.

REFERENCES

1. Weigert, C. Ausgedehnte umschriebene Miliartuberculose in grossen offenen Lungenarterienästen. *Virchows Arch. f. path. Anat.*, 1886, **104**, 31.

2. Thorel, Ch. Pathologie der Kreislauforgane. *Ergebn. d. allg. Pathol. u. path. Anat.*, 1903, **9**, Pt. 1, 1039.

3. Benda, C. Casuistische Mittheilungen über Endangitis tuberculosa mit Demonstration. *Verhandl. d. deutsch. path. Gesellsch.*, 1899, **2**, 335.
Die tuberkulösen Erkrankungen der Blutgefässe. *Ergebn. d. allg. Pathol. u. path. Anat.*, 1898, **5**, 452.

4. Jores, L. Herz und Gefässe. Handbuch der speziellen pathologischen Anatomie und Histologie, by Henke, F., and Lubarsch, O. Julius Springer, Berlin, 1924, **2**, 668.

DESCRIPTION OF PLATES

PLATE 5

FIG. 1. Drawing of heart and pulmonary artery showing three polypoid tuberculous vegetations attached to the wall of the pulmonary artery.

FIG. 2. Wall of the pulmonary artery at the commissural level with attached vegetation, illustrating the continuity of the tuberculous granulation tissue in the adventitia with that of the vegetation through an interruption in the media. Weigert's elastic tissue stain. × 20.

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Gross Tuberculous Vegetations of Pulmonary Artery

Plate 6

Fig. 3. Intima and a small portion of the media, showing a blood vessel in the intima surrounded by tuberculous granulation tissue. Hematoxylin and eosin stain. × 110.

Fig. 4. Tuberculous granulation tissue in the intima and hyaline swelling of the internal elastic lamina. Weigert's elastic tissue stain. × 134.

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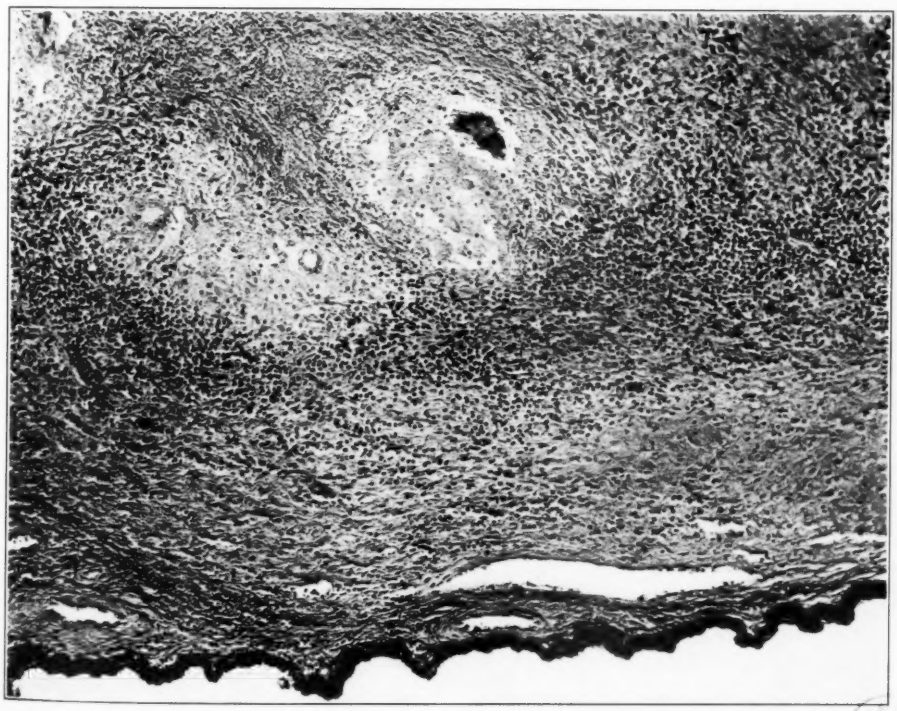

4

Gross Tuberculous Vegetations of Pulmonary Artery

MICROINCINERATION STUDIES OF HUMAN CORONARY ARTERIES *

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The method of microincineration of microscopic specimens of tissues devised by Liesegang was revived and improved by Policard, Tschopp, and by Scott. It has been employed for numerous studies in the microscopic anatomy of the distribution of salts in various parts of the body. Pathology has profited little as yet except for a few studies, notably those of Schultz-Brauns. Zinkant studied the arteries of the uterus by this method and showed what he believed to be the beginning of arteriosclerotic changes comparatively early in life. There is, however, reasonable doubt that the deposit of calcium within the arterial walls, as revealed by the method of microincineration, necessarily represents a pathological change. The present study deals with the ash left by the coronary arteries of the heart following microincineration, and considers the matter in reference to age periods.

Method and Materials

In general, the method as described by Policard was employed. The material was from 62 human hearts at different ages. From each heart four blocks were made. One block was in vertical and another in tangential section from the wall of the left ventricle midway between apex and base near the septum; one vertical section from the anterior border of the right ventricle near the septum and a tangential section just anterior to the margo acutus were also made. These blocks, from 1 to 2 cm. square and not more than 5 mm. thick, were taken before the heart was washed and placed immediately in a solution of 9 parts absolute alcohol to 1 part neutral formalin (40 per cent formaldehyde, C.P.). After not less than 24 hours' fixation the material was placed in absolute alcohol and, after about 6 transfers at 2 hour intervals, embedded in paraffin by the usual technique

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and cut in sections 6 microns thick. Five sections were made from each block. Sections 2 and 4 were stained with hematoxylin and eosin or orcein and hematoxylin. For sections 1, 3 and 5 slides were freed of grease with the utmost care, thoroughly cleaned and placed upon a warm stage at about 45° C. A few drops of alcohol were placed upon each slide and the section placed upon this alcohol. The warmth permitted the sections to flatten out smoothly and the material was then dried in air. In our experience water has proved to be quite as satisfactory as alcohol and does not appear to dissolve any of the salts out of the tissue. Incineration was carried out in a small electric oven so adjusted by rheostat that it maintained a temperature of 650° C. Incineration was continued until all charred material had disappeared and the sections lay as a white tissue upon the slide. The section was covered with a coverglass and sealed with paraffin. The examinations were with dark-field illumination, as suggested by Scott. The materials were grouped according to decades and the accompanying tables give an outline of the findings.

Results

First Decade: (Table I) This group included 10 cases ranging in age from newborn to 8 years. The gross findings as regards coronary arteries were negative throughout and this is true as regards microscopic findings, except in Case 10 in which typical rheumatic infiltrations were found in the myocardium and around the coronary vessels. In this decade the incinerated preparations appeared to show a smaller number of blood vessels than was observed in the hematoxylin and eosin preparations, but this dissimilarity was found to be due to the fact that no observable ash was produced by the very small vessels. In the position of the intermediate arteries there was found a ring of ash which in the younger children appeared as a row of fine beads, but in the older children as a continuous line. At times minute nodules appeared to project into the lumen of the vessel. This ring of ash is interpreted as representing the intima, more especially the endothelial cells of that coat. With the advance of age the line, which became continuous, also became increased in density. In the younger children the media and adventitia were not recognized by the ash, but in the older children a few small dots of ash were found in both coats. The exception to this was in that of Case

10 where the ash in adventitia and perivascular connective tissue was distinctly increased, apparently because of the cellular content in this region.

Second Decade: (Table II) Of the 4 cases in this group from 15 to 20 years of age, 2 showed slight beginning atherosclerosis of the coronaries grossly, and the other 2 showed no change. The sclerosis was almost completely confined to the larger branches, but in Case 4 of this group the smaller vessels in both ventricles showed slight intimal fibrosis. The principal difference discovered between this group and the patients in the first decade was the increase in amount of ash found in the intima. The adventitia and media left about the same amount of ash as in the older children of the preceding decade. In Case 4 mentioned above, a patient 20 years of age, the intima was distinctly richer in ash, and the media showed slightly more ash than in the other cases. A distinction between the ash of the three layers of the arterial wall was not easily possible.

Third Decade: (Table III) This group included 9 cases from 25 to 30 years of age. Grossly, all showed slight atherosclerotic change in the larger divisions, with the exception of Case 8 in which the artery appeared to be normal. With the hematoxylin and eosin preparations it was found that in 3 of these cases the sclerosis of the smaller arteries was confined entirely to the left ventricle, whereas in the other it was found in both ventricles. The intima in these cases showed moderate thickening, together with splitting and apparent multiplication of the internal elastic lamina. In 4 instances there was no manifestation of arteriosclerosis in the smaller vessels microscopically. In this age group it became possible to distinguish the ash of intima, media and adventitia. Generally speaking, the ash of the intima was greater in amount than in the preceding group and was especially prominent in the sclerotic areas. In 1 case without sclerosis the ash of the intima was definitely increased, as compared to earlier age groups, but the elastica stain preparation showed very clearly an increase in the amount of elastic tissue in the intimal coat. In certain of the arteries of Case 7, a leukemic patient, the ash appeared as tiny spots in the adventitia, corresponding to areas of leukemic infiltration. There were three female patients in this group and the amount of ash in the intima was definitely less than was true of the males.

TABLE I

First Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	Newborn	M	Hemorrhage of meninges and lungs	Negative	Arteries delicate. Ash even of larger intramuscular branches varies a little as a discontinuous ring, in media and adventitia as tiny spots
2	5 mo.	M	Bronchopneumonia	Negative	Arteries negative. Ash content as in preceding case
3	8 mo.	M	Spina bifida, acute purulent spinal meningitis	Negative	Small artery well contracted. Wall appears to be thickened. Intima of large artery gives a little more ash, less from media and adventitia
4	9 mo.	F	Influenza meningitis	Negative	Ash of intimal layer as a sharp ring with a little irregular outline
5	11 mo.	M	Embryonic tumor of left kidney	Negative	Ash of intimal layer as a sharp ring with a little irregular outline
6	3 yrs.	M	Acute purulent meningitis	Negative	No pathological changes. Ash in three layers of larger artery slightly increased
7	3½ yrs.	M	Enteritis follicularis	Negative	No pathological changes. Ash in intima of large arteries as a ring with slight irregularity in outline Very little ash in media and adventitia

8	5 yrs.	M	Bronchopneumonia	Negative	No pathological changes. Ash in intima of large arteries as a ring with slight irregularity in outline. Very little ash in media and adventitia
9	6 yrs.	M	Acute purulent meningitis	Negative	No pathological changes. Ash in intima of large arteries as a ring with slight irregularity in outline. Very little ash in media and adventitia
10	8 yrs.	F	Chronic rheumatic endocarditis	Negative	In adventitia and perivascular region of intramuscular arteries often infiltration of large and small mononuclear cells is seen. Ash in media and adventitia of some large arteries increased as tiny spots. Ash in small artery as distinct ring

TABLE II

Second Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	*years* 15	F	Poliomyelitis, bronchopneumonia	Negative	Internal elastic lamella of intima very well developed, especially on left side. All intramuscular arteries give distinct ash ring in intima
2	17	M	Chronic ependymitis, hydrocephalus	A few small atheromatous patches in the left anterior descending branch. Right coronary artery negative	Slight proliferation of elastic fibers in intima of a few intramuscular arteries of left ventricle. Ash picture about the same as in preceding case
3	18	M	Chronic tuberculosis of lungs and spine	Negative	Intima of arteries no change, gives quite distinct ash ring
4	20	M	Generalized tuberculosis	In intima of beginning portion of left anterior descending branch a few yellowish streaks. Right coronary artery negative	Thickening and fraying out of internal elastic lamella of arteries of some trabecular muscles of both ventricles. Ash of large arteries somewhat increased in every layer, especially in intima of diseased arteries

TABLE III

Third Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	*years* 25	M	Cerebral hemorrhage, bronchopneumonia, cardiac hypertrophy	Atheromatous patch in the intima of the left anterior descending branch. Right coronary artery negative	Slight patchy thickening of intima and internal elastic lamella of a few intramuscular arteries, giving increased ash of small arteries, very distinct in intima
2	26	M	Chronic ulcerative pulmonary tuberculosis	A number of atheromatous patches in left anterior descending branch. A few minute yellow spots in right coronary artery	Slight thickening and fraying out of internal elastic lamella of a few middle-sized arteries. Ash in intima of arteries quite distinct
3	27	M	Lobar pneumonia	In left circumflex a yellow spot. Right coronary artery negative	Patchy thickening of a large artery of left ventricle. Increase of adventitial tissue. Ash increased in all layers of large artery. Ash in intima of arterioles very distinct
4	28	M	Bilateral lobar pneumonia	Left anterior descending artery shows slight thickening of the intima around an orifice of its branch. Right coronary artery negative	Internal elastic lamella of arteries very well developed. Ash found chiefly in intima. Little ash from media and adventitia
5	29	M	Acute mastoiditis, purulent cerebrospinal meningitis	Left coronary artery shows slight patchy thickening of the intima. Right similar to left	Patchy thickening of intima of a few large and small arteries with increased elastic fibers. Ash in intima as a rather thick, irregular ring. Ash in media of large artery also increased

TABLE III (*Continued*)

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
6	*years* 29	M	Ulcerative pulmonary tuberculosis	An atheromatous patch in the intima of the left anterior descending artery. Right the same as left	Changes similar to those of Case 4
7	29	F	Chronic myeloid leukemia	Left coronary artery shows patchy thickening of intima in some places. Right coronary artery shows similar changes, but to a lesser degree	Wall of arteries thin and delicate. Ash of intima little in amount, but in media and adventitia increased as numerous tiny spots
8	29	F	Chronic pulmonary tuberculosis	Both coronary arteries negative	Wall of arteries thin and delicate. Ash of intima slight in amount
9	30	F	Purulent osteomyelitis, streptococcus sepsis	In both coronary arteries a few atheromatous patches	Similar to Case 8

TABLE IV

Fourth Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	*years* 34	M	Bronchopneumonia	Left coronary artery shows a few atheromatous patches found in anterior descending branch. Right coronary artery shows numerous fine yellow spots	Arteries show no definite sclerotic changes in hematoxylin-eosin and elastic preparations. Ash in wall of medium-sized and smaller arteries as more or less irregular, quite clear ring. Ash in media of large artery very slight
2	35	F	Infectious encephalomyelitis	Slight thickening of intima around some orifices in left anterior descending artery. Right coronary artery the same	Intima of a few large arteries of left ventricle slightly thickened. Ash picture similar to that of preceding case
3	36	M	Generalized tuberculosis	In both coronary arteries a few atheromatous patches	No arteriosclerotic changes. Ash in small arteries as distinct ring
4	37	M	Gangrene of lower extremities	A few atheromatous patches in left anterior descending artery. Many in the right coronary artery	A few middle-sized and small arteries of left ventricle showing thickening of intima and proliferation of elastic fibers, giving a thick ash ring or with frayed-out processes, or ring with double contour. Changes of artery of right ventricle similar
5	38	M	Bronchopneumonia	A few atheromatous patches in beginning portion of left anterior descending branches. Right coronary artery negative	Many arteries show thickening of intima, especially large arteries. Proliferation of elastic fibers in intima. Ash considerably increased in intima, also in media and adventitia
6	39	M	Generalized tuberculosis	Many fine yellow spots in intima of anterior descending branch of left coronary artery. Right coronary artery similar to a lesser degree	Similar to Case 3

Fourth Decade: (Table IV) In this group there were 6 cases from 34 to 39 years of age. In all cases the gross examination showed varying degrees of atherosclerosis in the larger branches of the coronary arteries. The ash of the intima as compared with the preceding age group was not only greater in amount but showed increasing density. In some of the cases the delimitation between intima and media was obscured because of what appeared to be a "fraying out" of the ash into the intima at points where the ash in the two coats could not be distinguished. Generally speaking, the ash of media and adventitia was clearly separable and appeared as a delicate network in both coats, increased in amount over the age group preceding. In Case 5 the amount of ash in all three coats was definitely greater than in the other members of this group.

Fifth Decade: (Table V) This group was made up of 14 cases ranging in age from 42 to 50 years. The gross examination showed definite intimal atherosclerosis in 12 of these 14 cases, and in Case 5 this was particularly prominent. Microscopically, with the hematoxylin and eosin stains, sclerotic changes were observed in smaller vessels of all these cases. These changes were, as a rule, distinctly more marked in the left ventricle than in the right. The ash from the arteries of all these cases was increased from all three coats as compared with the preceding age period, except in Case 9. In this instance, where there was no arteriosclerosis grossly and only slight sclerosis microscopically, the ash of the intima formed a thin, delicate ring and the ash of adventitia and media was very slight in amount. In those small divisions where sclerosis was prominent, the ash of intima showed a corresponding irregularity in relation to the lumen of the vessel.

Sixth Decade: (Table VI) Of the 10 cases of this group, ranging in age from 52 to 60 years, all showed noteworthy gross atherosclerosis of the coronaries and in 5 of the cases this was accompanied by small areas of calcification. The hematoxylin and eosin preparations showed varying degrees of intimal sclerosis in the smaller branches, but these changes were not parallel to those observed in the large divisions. In this group and in the preceding group sclerotic changes in the smaller vessels were observed, especially in the branches to the trabeculae carneae. In general the ash of the intima was considerably increased in amount over the preceding age group, corresponding closely to the sclerosis present in the vessels studied. In some of

the small arteries the amount of ash was so great that the lumen appeared to be almost occluded. The media and adventitia both showed a large quantity of ash. In many instances the perivascular connective tissue showed a delicate network of ash corresponding in general to the distribution of the loosely arranged collagenous fibers. With the dark-field illumination the ash of the perivascular tissue was of a pale blue color, as compared with the white of the coat of the vessels. In 1 case, illustrated in Figure 7, the ash of intima was in the form of delicate, radiating bands corresponding to the arrangement of elastica in that coat.

Seventh Decade: (Table VII) The 5 cases in this group were 61 to 70 years of age. The findings were so closely similar to those in the sixth decade that no separate description seems justified. The changes were essentially the same in all respects, except that an impression was gained that the amount of ash was somewhat increased and slightly more irregularly distributed.

Eighth Decade: (Table VIII) Of the 5 cases included in this group, 71 to 79 years of age, all showed definite coronary sclerosis grossly. Again the changes found in the smaller divisions were not parallel to those found in the larger arteries. In the hematoxylin and eosin preparations the intima showed nodular thickening in many instances and the amount of elastica in the patches was increased, as compared with the rest of the intima. The incinerated specimens showed a definite increase of the ash in the walls of almost all the arteries and this was especially marked in the smaller vessels. In these the ash of the intima was characterized by irregularities, apparently due to the patchy thickening. The ash of media, adventitia and perivascular connective tissue was markedly increased, quite regardless of the degree of sclerosis.

Discussion

The incinerated preparations show ash from the coronary arteries in all the ages included in this study. In young subjects the ash of small branches may be so small in amount that it does not form a continuous ring. In the first three decades there is a progressive increase in the amount of ash, but this does not in all instances parallel the advance of age. Exceptions are especially noteworthy in the females of the third decade. Limitations of time and material made it impossible to distinguish as to sex differences, but it is assumed

TABLE V

Fifth Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	*years* 42	M	Cellulitis of scrotum and pelvic tissue	Slight thickening of intima around orifices of branches of left anterior descending artery. The same in the right coronary artery	Some large and small arteries with patchy thickening of intima and increase of elastic fibers of internal elastic lamella. In hematoxylin and elastic stains changes of artery of right ventricle definitely less in degree. A great deal of ash found in intimal layer. Little in media and adventitia of large artery
2	44	M	Generalized arteriosclerosis, luetic aortitis	Slight thickening of intima around orifices of branches of left anterior descending artery. The same in the right coronary artery	Similar to Case 1
3	44	M	Generalized tuberculosis	A few atheromatous patches in intima of left anterior descending branch. Right coronary artery shows only one yellowish patch	Slight patchy thickening of intima of large artery. Ash in large and small arteries little in amount
4	45	M	Acute toxic hepatitis	Left coronary artery slightly stiff, showing some patchy thickening of its intima	Slight arteriosclerotic changes of large arteries. Considerable amount of ash, especially in intima of arteries
5	46	F	Hypertension, syphilitic aortitis	Marked arteriosclerosis with stiffness and atheromatous changes of coronary arteries, more on left side	Considerable thickening of intima with increase of elastic fibers in all arteries, especially those of left ventricle. Ash much increased in all layers of artery, especially the intima. Little ash also increased in perivascular fibrous tissue

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6	46	M	Generalized tuberculosis	In beginning portion of left anterior descending artery numerous atheromatous patches. Right coronary artery free	Thickening of intima and increase of elastic fibers of internal elastic lamella of most arteries, especially small ones. Artery of left ventricle shows more marked changes than that of right. Ash increased in all layers
7	46	M	Lobar pneumonia	A few atheromatous patches in left anterior descending branch. Right coronary artery same changes	Changes similar to those of Case 6, but to a higher degree
8	46	F	Bronchopneumonia, generalized arteriosclerosis	In beginning portion of left anterior descending branch a few atheromatous patches. Right coronary artery shows numerous confluent yellow patches. Same thing in circumflex artery	Much thickening of intima of arteries, narrowing of their lumina. Thickening of intima chiefly due to increased fibrous tissue. Elastic fibers of internal elastic lamella slightly increased. Two kinds ash: faintly illuminated in fibrous part of intima and brightly illuminated in elastic part
9	47	M	Bronchopneumonia	Both coronary arteries negative	All thin-walled arteries giving ash in form of a delicate ring
10	47	F	Cancer of breast with extensive ulceration	Both coronary arteries negative	Similar findings as in Case 1
11	48	M	Bronchopneumonia	Slight thickening of intima around orifices of branches of left anterior descending branch. Right coronary artery same change	No arteriosclerotic changes found, but in intima a distinct ash-ring is observed
12	49	F		A few minute yellow spots in beginning portion of left anterior descending branch. Right coronary artery negative	Slight thickening of a few large and middle-sized arteries. Ash picture similar to that of Case 11

TABLE V (*Continued*)

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
13	*years* 50	M	Chronic pulmonary tuberculosis; cardiac hypertrophy and dilatation	A few atheromatous patches in large branches on left side. Right coronary artery shows one atheromatous patch in its beginning portion	Elastic fibers of intima of some intramuscular arteries considerably increased. Ash of small arteries as a thick ring
14	50	M	Cerebral hemorrhage; arteriosclerosis of aorta; cardiac hypertrophy and dilatation	A few atheromatous patches in beginning portion of left anterior descending branch. Right coronary artery stiff with marked atheromatous changes	Small arteries, especially in the trabecular muscles of both ventricles, arteriosclerotic with increased elastic fibers in intima. Ash of such arteries as a thick ring irregular in outline. Large and middle-sized arteries changed in similar manner, but less marked

TABLE VI

Sixth Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	*years* 52	M	Chronic pulmonary tuberculosis, cardiac hypertrophy and dilatation	A few atheromatous patches in left anterior descending branch. Right coronary artery the same	Large and middle-sized arteries slightly arteriosclerotic, but small ones show severe changes with obliteration of lumina by reproduction of elastic fibers in intima. Ash of such arteries greatly increased
2	52	F	Generalized arteriosclerosis, cardiac hypertrophy and dilatation	Lumina of large branches of left coronary artery much narrowed, with numerous calcified patches. Right coronary similar to left	Intramuscular arteries of deeper layer of left ventricle quite arteriosclerotic with increased ash amount. Arteries of superficial layers and of right ventricle slightly changed
3	54	F	Generalized arteriosclerosis, cardiac hypertrophy and dilatation	Large branches of left coronary artery almost diffusely occupied by atheromatous and calcified patches. Some hyaline plaques and atheromatous patches in right coronary artery	Considerable degree of arteriosclerosis of a number of intramuscular arteries. Ash considerable in amount in intima of such arteries
4	54	F	Cancer of breast	Left coronary artery, beginning portion of anterior descending branch shows an atheromatous patch. Right coronary artery negative	Pronounced arteriosclerosis almost entirely limited to arteries of trabecular muscles of left ventricle with increased ash in intima. Slight increase of ash in media of large and middle-sized arteries
5	55	M	Chronic pulmonary tuberculosis	A few small atheromatous patches in left anterior descending branch. Right coronary artery shows a large atheromatous patch	Similar changes as in preceding case

TABLE VI (*Continued*)

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
6	*years* 55	M	Gangrenous cystitis, pyonephritis	Thickening of wall and narrowing of lumen of all coronary arteries. Intima with numerous atheromatous and calcified patches. Changes on left side more marked	Moderate degree of arteriosclerosis of intramuscular arteries of deeper layer of myocardium of left ventricle, less of right ventricle. Ash in intima increased, same in media and adventitia of larger arteries
7	58	M	Spinal cord tumor; generalized arteriosclerosis	Coronary arteries *on both sides* stiff and their lumina narrowed with many atheromatous calcified patches	Similar to preceding case
8	58	M	Cancer of esophagus	Numerous atheromatous and calcified patches in intima of beginning portion of left anterior ascending branch. Right coronary artery similar to left	Intramuscular arteries of deeper layer of myocardium of left ventricle greatly thickened. Elastic fibers much increased. Ash in all layers, especially in intima, increased
9	58	M	Generalized arteriosclerosis and lobar pneumonia	Marked atheromatous changes of intima of both coronary arteries	Marked arteriosclerotic changes of all arteries, especially small arteries. Ash increased in three layers
10	60	M	Syphilitic aortitis, generalized arteriosclerosis	Slight thickening of intima of left anterior descending artery around orifices of its branches. Right coronary artery negative	Moderate arteriosclerosis of middle and smaller-sized arteries of left ventricle. Arteries of right ventricle negative except for a few small ones. Ash of intima of small arteries slight in media, and in adventitia of large and middle-sized arteries increased

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TABLE VII

Seventh Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	*years* 61	M	Arteriolar nephrosclerosis, cardiac hypertrophy and dilatation	Thickening and atheromatous changes of intima around orifices of branches of both coronary arteries	Considerable degree of arteriosclerosis of all intramuscular arteries. Thickening and reproduction of elastic fibers of intima of trabecular arteries in left ventricle marked. Ash in intima increased
2	62	M	Tabes dorsalis, syphilitic aortitis, bronchopneumonia	Left anterior posterior descending and circumflex artery shows many atheromatous and calcified patches in intima. Right coronary artery, similar changes, but limited to beginning portion	Fairly marked arteriosclerotic changes of all arteries of both ventricles. Ash findings similar to those of preceding case
3	65	M	Chronic pulmonary tuberculosis	Coronary arteries on both sides show some hyaline patches around orifices of their branches	Marked arteriosclerosis of small arteries of trabecular muscles of both ventricles. Ash increased in small arteries, also slight amount in media and adventitia of middle and large arteries

TABLE VII (*Continued*)

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
4	*years* 67	M	General arteriosclerosis and thrombosis of both coronary arteries	Left coronary artery with marked atheromatous changes in all large branches. Calcified patches of anterior descending branch and old thrombus in its beginning portion. Right coronary artery shows similar changes and fresh thrombus in its beginning portion	Marked arteriosclerosis, chiefly of large and middle-sized arteries with increase of elastic fibers and connective tissue in intima. Changes of artery of left ventricle more marked. Increased elastic fibers in adventitia and perivascular region. Ash increased in amount, especially in intima
5	70	M	Cancer of bladder	Marked arteriosclerosis of coronary arteries on both sides with numerous calcified and atheromatous patches in intima, their intima being greatly narrowed. Occlusion of left anterior descending branch	Similar changes as in Case 4, but to a lesser extent

TABLE VIII

Eighth Decade

Case No.	Age	Sex	Anatomical diagnoses	Macroscopic findings of coronary arteries	Microscopic findings of coronary arteries
1	*years* 71	M	Coronary thrombosis, right	Stiffness and narrowing of all large coronary branches showing numerous atheromatous and calcified patches, especially in left anterior descending branch. Thrombosis of right coronary artery	Arteries generally thick-walled with increased amount of elastic fibers in intima, in certain places also in perivascular region. Ash increased considerably in intima
2	72	M	Carcinoma of gall bladder	Numerous atheromatous patches in left anterior descending branch. Right coronary artery negative	Marked thickening of intima of a few intramuscular arteries with much increase of elastic fibers in intima. Ash in intima and media rather increased
3	73	M	Generalized arteriosclerosis, encephalomalacia	Many atheromatous patches, some of them calcified, especially in left anterior descending branch. Right coronary artery much narrowed	Marked arteriosclerosis of small and middle-sized arteries. Internal elastic lamella greatly increased in thickness. Lumen much narrowed. Increased amount of ash in intima
4	75	M	Bronchopneumonia	Many atheromatous and calcified patches of left coronary artery. Right coronary artery similar, but to a lesser degree	Advanced arteriosclerosis of small arteries of both ventricles. Internal elastic lamella thickened, chiefly in patchy form and frayed out. Ash increased, especially in intima
5	79	M	Generalized arteriosclerosis, cardiac hypertrophy and dilatation	Numerous atheromatous and calcified patches in intima of large branches of left coronary artery. Wall stiff and lumen narrowed. Right coronary artery with many atheromatous patches	Most intramuscular arteries show thickening of internal elastic lamella, usually partially button-like, protruding into lumen. Changes on left side more prominent. Ash of intima increased chiefly in arteriosclerotic arteries

that generally the coronaries of the female probably show less ash than those of the male. The increase in amount of ash affects all the arteries studied. After the third decade, although there is a progressive increase in amount of ash, the progression lacks the regularity observed in the first three decades. The greatest amount of ash was observed not in the eighth decade, but in a 58 year old male patient. The variation between arteries of the same case in these later age periods is greater than in the earlier periods. In the sixth decade the greatest amount of ash was found in those arteries supplying the trabeculae carneae. In the seventh and eighth decades the increase in amount of ash affected all the arteries studied. Thus, there is considerable variation in the increase in ash in these last three decades. The confusion is produced principally because of the varying degrees of arteriosclerosis, which in this series of cases was found to be most marked in the sixth decade.

The factor of arteriosclerosis is of much less significance in the first three decades and it would appear that the increase in amount of ash in these periods is parallel to the rate of growth of the body. In Case 12 of the first decade the pathological changes noted are responsible for the variation in ash content. Zinkant in his study of uterine arteries found no ash in these vessels of most of his cases in the first decade. There was a gradual increase of ash during the second decade. This he regarded as a deposit of calcium and probably incorrectly, as indicated below, interpreted it as a pathological change resulting from the functional alterations in the organ, a precursor of arteriosclerosis. Schultz-Brauns found ash in the aorta of a newborn and of a young child, but did not discuss its significance.

The present study indicates that the greatest amount of ash is in that part of the artery rich in elastic fibers. Figure 10 shows the position of ash in the media of the aorta especially rich in elastica. When compared with the renal artery (Fig. 11) which is a muscular type of vessel, the latter shows the ash especially about the internal elastic lamina. In the coronary arteries the ash is noteworthy in the intima, but generally is in comparatively small amounts in media and adventitia. The increase observed in the coronary arteries during the first three decades corresponds to the normal growth of the arteries and the "aufsteigende Periode" of Jores. Thus, a certain amount of ash must be considered as normal and not of necessity an indication of sclerosis, except in so far as sclerosis may be looked

upon as a process of aging. In these earlier decades where sclerosis is not demonstrable, the ash occurs in fairly regular ring or oval forms, but when sclerosis becomes noteworthy the ash lacks uniformity in arrangement, is often eccentric in distribution and variable in quantity. It is probable that the same argument explains the small amount of ash in the coronaries of the female. Since the largest amount of ash of media was found in the last decade it is assumed that the increase in ash of media is a function of age, which may be explained by the demonstration of Bürger and Schlomka that any tissue of older persons becomes increased in density.

As indicated by Policard and Schultz-Brauns, it is generally agreed that the ash is made up principally of calcium, which in the incinerated preparation appears as calcium oxide. Treatment of the specimen with 2 per cent sulphuric acid produces the typical gypsum crystals of calcium sulphate, as shown in Figure 12. The material takes the hematoxylin stain as does calcium, and stains by the method of von Kossa. It is of great importance in the technical procedure that the incineration be conducted at 650° C until all organic and volatile material is removed. This can be controlled by washing with water, because if the amount of ash which remains after washing is essentially the same as before washing, it can be regarded as pure ash. As demonstrated by Policard, other minerals such as iron and magnesium are present, but these are in such small amounts that in all likelihood they can be disregarded.

As can be seen in the accompanying photomicrographs there is no doubt that muscle and connective tissue leave ash after incineration and the ash may be regarded as representing normal constituents of these tissues, as shown by Scott and by Schultz-Brauns. As indicated by the results shown in Figures 10 and 11 there is no reasonable doubt but that the elastic tissue leaves a considerable amount of ash. The origin of the elastic fibers is uncertain. Jores believed the elastica to represent the differentiation of connective tissue cell products, whereas Hueck assumed it to come from the ground substance of the connective tissue, especially that of the intima of arteries. According to Wells, hyaline substances have an affinity for calcium salts, but in spite of the possibility of adsorption of calcium, it nevertheless seems probable that normal elastic tissue contains calcium in larger quantities than other tissues. Hofmeister and Henkel maintained that compounds of calcium salts and protein do not exist in

the body. The salts are kept in solution by protecting colloids and thus the normal content of the elastica in calcium would represent only a larger concentration of calcium salts in this tissue. As is well known, such combinations are unstable and subject to precipitation by changes in hydrogen ion concentration and perhaps other physical states of the tissue. The demonstrations by Ribbert, by Klotz, and by Faber of small amounts of precipitated calcium in the arteries of young persons may be the result of precipitation due to changes of the character indicated. In outspoken intimal arteriosclerosis, where an increase in elastica can be established, it may very well be that the calcification in precipitated form depends in large part upon the high concentration of calcium in the mass of elastic tissue. Calcium salts, however, may be precipitated in the region of elastica without any preceding increase of elastica, as is true of the isolated calcification of the internal elastic membrane described by Jores. The hypothesis given above does not serve to explain all types of calcification because there are noteworthy exceptions. For example, calcification may occur in tissues in which there is little or no elastic tissue. Conversely, as in mural endocardium where elastic tissue is rich in amount, calcification may be of uncommon incidence. In idiopathic medial necrosis of the aorta of Erdheim, calcification is rare in spite of the large number of elastic fibers in this situation. The marked medial calcification in Mönckeberg sclerosis is in muscular arteries with relatively little elastica. Even in the cases reported herewith, the coronaries often showed marked hyperplasia of the elastica without any noteworthy precipitation of calcium salts. It may therefore be stated that increase in the amount of elastica alone is by no means an indication that precipitation of calcium salts will occur. The facts, however, that the elastic fibers probably contain a large amount of calcium, and that calcification in the early stages of arteriosclerosis takes place in the area where the internal elastic membrane of the artery is located, lead to the assumption that the elastic fibers play an important rôle in the calcification of arteriosclerosis.

Summary and Conclusions

1. The method of microincineration was employed in the study of the smaller divisions of the coronary arteries in 62 cases ranging in age from newborn to 79 years.

2. Ash was demonstrated in all instances, but in variable amounts. The smallest amount was found in the arteries of newborn infants. This amount gradually increased during the first, second and third decades, apparently as a corollary of normal growth. The increase in amount of ash after the third decade was definite, but was without regularity of progress. This total variation, associated with individual variation, was probably the result of arteriosclerosis in these higher age groups. It was found also that rheumatic fever nodules, and leukemic infiltrates increased the amount of ash.

3. The ash of coronary arteries is derived principally from the elastic fibers, but also in small part from the intima, media and adventitia. This should be regarded as a normal finding, but not of necessity as a precursor of arteriosclerosis.

4. The ash is composed almost entirely of calcium salts, but the manner in which these salts are bound to the elastic fibers is not known.

5. As the quantity of elastic tissue increases normally and as it increases as a part of arteriosclerosis there is an increase in the amount of ash.

6. It is suggested but not proved that alteration in quantity and quality of elastica may have some bearing upon the calcification that occurs in the course of arteriosclerosis.

Indebtedness is expressed to Prof. H. T. Karsner who suggested this study, to Dr. David Seecof who kindly supplied part of the material, and especially to Dr. G. H. Scott of Washington University for invaluable aid as regards technique and other features of the work.

BIBLIOGRAPHY

Bürger, M., and Schlomka, G. Ergebnisse und Bedeutung chemischer Gewebsuntersuchung für die Alternsforschung. *Klin. Wchnschr.*, 1928, **41**, 1944.

Faber, A. Die Arteriosklerose. G. Fischer, Jena, 1912.

Henkel, K. O. Die Mikroveraschung. Handbuch der biologischen Arbeitsmethoden, 1929, **5**, pt. 2, No. 13, 1471.

Hofmeister, F. Experimentelles über Gewebsverkalkung. *München med. Wchnschr.*, 1909, **38**, 1977.

Jores, L. Arterien. Die Genese der postembryonalen Intima-entwicklung. Handbuch der speziellen pathologischen Anatomie und Histologie, Henke, F., and Lubarsch, O. J. Springer, Berlin, 1924, **2**, 690.

Jores, L. Über die feineren Vorgänge bei der Bildung und Wiederbildung des elastischen Bindegewebes. *Beitr. z. Anat. u. z. allg. path.. Pathol.*, 1907, **41**, 167.

Klotz, O. Studies upon calcareous degeneration. I. The process of pathological calcification. *J. Exper. Med.*, 1905, **7**, 633.

Liesegang, R. E. Die Veraschung von Mikroschnitten. *Biochem. Ztschr.*, 1910, **28**, 413.

Policard, A. Sur une méthode de micro-incinération applicable aux recherches histochemiques. *Bull. Soc. chim. de France*, 1923, **33**, 1551.

Policard, A., and Okkels, H. Localizing inorganic substances in microscopic sections. *Anat. Record*, 1930, **44**, 349.

Ribbert. See Jores, (Arterien.)

Schultz-Brauns, O. Histo-topochemische Untersuchungen an krankhaft veränderten Organen unter Anwendung der Schnittveraschung. *Virchows Arch. f. path. Anat.*, 1929, **273**, 1.

Scott, G. H. Disposition of the fixed mineral salts during mitosis. *Bull. d'histol. appliq. à la physiol.*, 1930, **7**, 251.

Scott, G. H. Distribution of mineral ash in striated muscle cells. *Proc. Soc. Exper. Biol. & Med.*, 1932, **29**, 349.

Tschopp, I. Die Lokalisation anorganischer Substanzen in den Geweben (Spodographie). Handbuch der mikroscopischen Anatomie, 1929, **1**, pt. 1, 571.

Wells, H. G. Chapter in Survey of Arteriosclerosis. Josiah Macy, Jr. Foundation. (In press.)

Zinkant, W. Histo-topochemische Untersuchungen über die Schwankungen des Kalkgehaltes in den Arterien des Uterus. *Virchows Arch. f. path. Anat.*, 1931, **281**, 911.

DESCRIPTION OF PLATES

PLATE 7

All photomicrographs taken with dark-field illumination.

FIG. 1. Case 5, Table I. 11 months old male. Medium-sized deep branch of left coronary. Ash forms complete ring, without distinction of three layers. × 150.

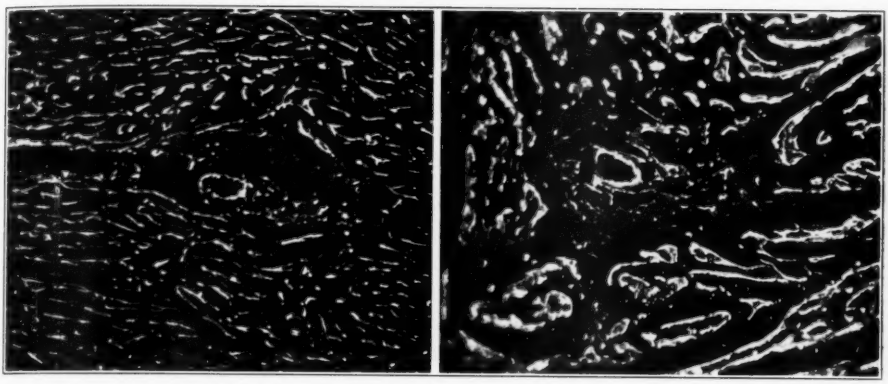
FIG. 2. Case 4, Table II. 20 year old male. Small deep branch of left coronary. Suggestion of division of ash into three layers. × 150.

FIG. 3. Case 8, Table III. 29 year old female. Two small deep branches of left coronary. Suggestion of distinction into three layers. × 150.

FIG. 4. Case 2, Table IV. 35 year old female. Medium-sized branch of right coronary artery. Ash of intima distinguished from smaller amounts of ash in media and adventitia. × 150.

FIG. 5. Case 13, Table V. 50 year old male. Medium-sized branch of right coronary. Irregular distribution of ash of sclerotic intima. Ash of media and adventitia slight in amount. × 150.

FIG. 6. Case 8, Table VI. 58 year old male. Small deep branches of right coronary. Large amount of ash from sclerotic intima. Decrease in size of lumina. × 150.



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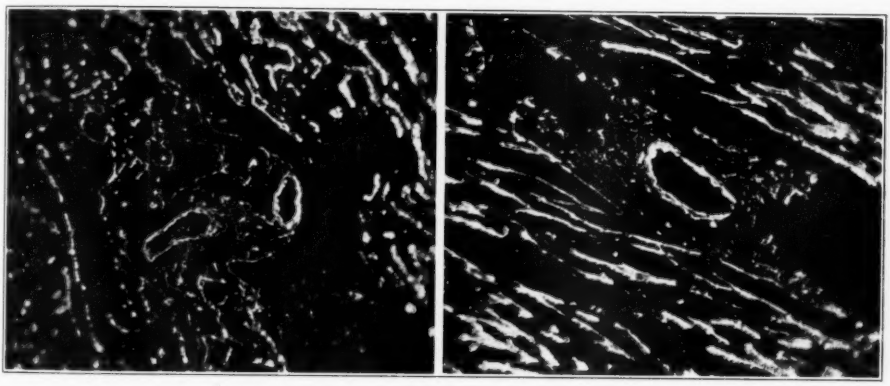

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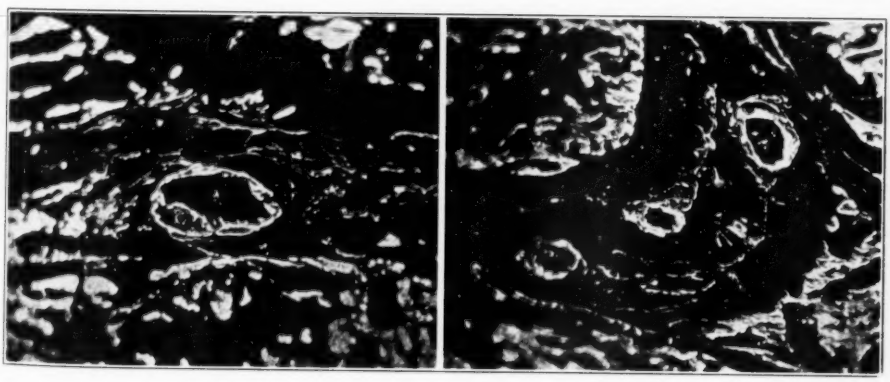

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Ku Microincineration Studies

PLATE 8

FIG. 7. Case 10, Table VI. 60 year old male. Medium-sized branch of left coronary. Radiating arrangement of ash of sclerotic intima. × 150.

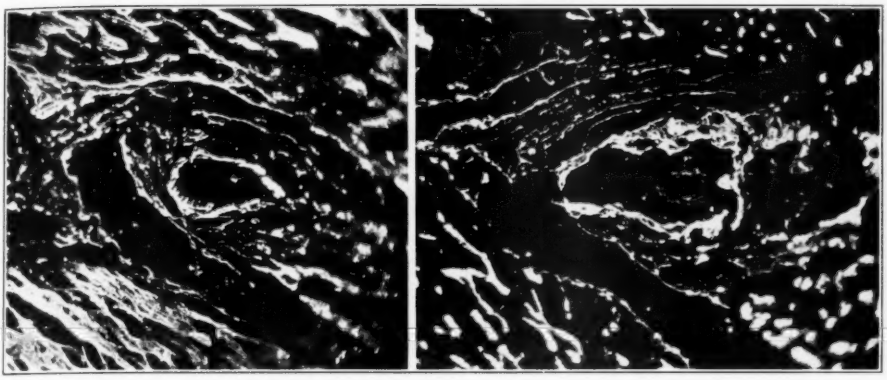
FIG. 8. Case 5, Table VII. 67 year old male. Medium-sized branch of right coronary. Inner layer of ash chiefly from intima. Note density and irregular distribution of ash. × 150.

FIG. 9. Case 3, Table VIII. 73 year old male. Small branch of right coronary. Ash of a non-sclerotic artery. Note fairly heavy deposit of ash of media and adventitia. × 150.

FIG. 10. Ash from normal aorta of a 34 year old male. The ash is principally in the media and appears to follow lines of elastica. The ash of intima is small in amount and that of adventitia still less. × 70.

FIG. 11. Ash from normal renal artery of a 34 year old male. Note the ash of the internal elastic membrane. The adventitia shows less ash than the media. × 70.

FIG. 12. Gypsum crystals of calcium sulphate in incinerated specimen of aorta, following treatment with 2 per cent solution sulphuric acid. Above is the ash in media, below are the needle-shaped crystals, and between, a clear zone where the calcium salts are in solution. × 150.



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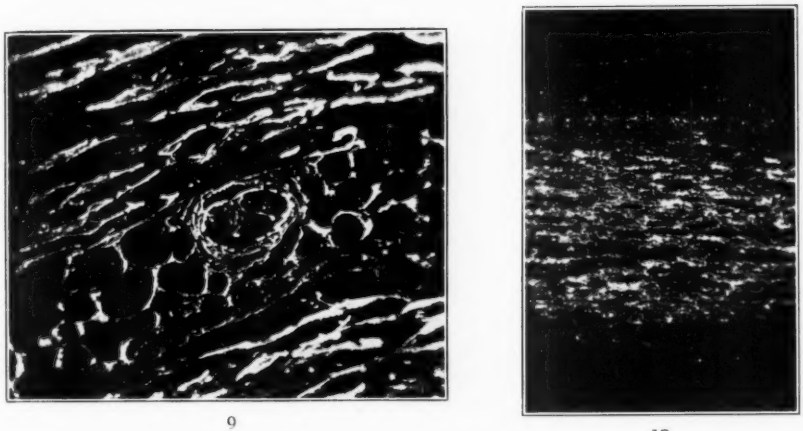

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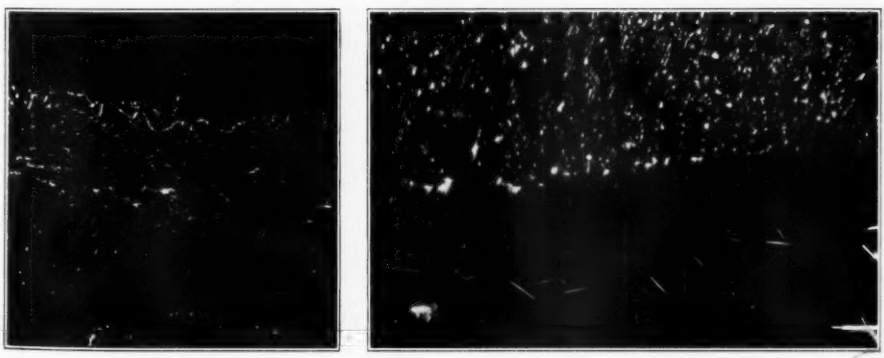

11 12

EXPERIMENTAL FAT EMBOLISM OF THE HEART *

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In spite of numerous studies, opinions regarding the importance of fat embolism remain divided. Lehman and Moore [1] in a recent review were unable to find agreement on any phase of the problem. In previous discussions [2] it is repeatedly stated that fat embolism may cause death by extensive obstruction of capillaries, either in the lungs or in the brain. The pulmonary type has a rapid course with dyspnea, cyanosis, cough and signs of pulmonary edema predominating; the cerebral type has a longer course and is characterized by stupor, coma, paralyses, sphincter disturbances and so on, and by evidence of embolism of the systemic circulation, such as petechial hemorrhages in the skin, free fat globules in the retinal vessels and fat in the urine.

Other than solely mechanical effects have also been suggested. Gold and Löffler,[3] working with cats, concluded that when posttraumatic fat embolism is slight death is due to simultaneously appearing shock, whereas if a large amount of fat enters the circulation death results with symptoms resembling shock. In this connection the work of Porter [4] on the production of shock by fat embolism is of interest. Paul and Windholz,[5] on the basis of postmortem and experimental studies, concluded that death in the cerebral form is due to injury of the brain, liver and kidneys, accompanied by symptoms of uremia. Lehman and Moore suggest that as there is little correlation between the severity of the symptoms and the amount of fat found after death physiochemical changes in the blood plasma may play an important rôle, especially as fat embolism sometimes occurs without trauma. Weimann,[6] on the other hand, insists that changes in the brain in this form of embolism are sufficient to cause death without invoking "autotoxic processes in other organs" to explain it.

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In an extensive review Vanucci and Franceschini [7] conclude that none of the theories — pulmonary, cerebral, renal or cardiac — adequately explain death. Although they believe that fat embolism alone can cause death in man, the part played by the fundamental disease, as well as shock accompaning injury, must be carefully considered. They also make the interesting statement on the basis of physiological experiments upon rabbits that "alterations of the function of the heart occur sooner, are more profound and more lasting than those of respiration." As Warthin remarked in 1913,[2] the importance of damage to the heart in fat embolism is strangely overlooked by most students of the problem. He noted that Colley and a few other observers are the only ones who have regarded the part played by the heart as of any consequence. Warthin observed fat emboli in branches of the coronary arteries with fatty changes in the heart muscle about the plugged capillaries and arterioles. He was of the opinion that the fat droplets in the muscle fibers were derived chiefly from the emboli and only a small portion of them from invisible fat in the cells. He was greatly impressed with the effect of such changes and insisted it was proper to speak of a *cardiac-symptom complex* in fatty embolism. He stated that this is accompanied by a great fall in arterial pressure, and a corresponding rise in the venous, the heart rate being very rapid and irregular. Since Warthin's contribution others have also considered the possibility of cardiac failure in fat embolism,[7] and have described changes in the muscle cells of the heart [8] similar to those Warthin found. We have thought that possibly some additional light on the problem of the part played by embolism of the vessels of the myocardium would accrue from studying this phase of fat embolism experimentally.

Expfrimental Methods

We used dogs and the method described by Rukstinat.[9] Under ether anesthesia the thoracic cavity was opened in the left third or fourth interspace under conditions preventing infection. While the pleural cavity was open, respiration was maintained by intratracheal insufflation of air and ether. After widening the aperture with a self-retaining retractor and packing away the lower lobes of the left lung from the surface of the pericardium, the coronary arteries were exposed by a vertical incision in this sac. Into one or more of the de-

scending branches of the front coronary artery 1 to 2 cc. of dog oil were slowly injected by means of a bent hypodermic needle attached to a 2 cc. syringe. The oil was obtained by mechanically expressing the omental and subcutaneous fat of normal dogs without the use of heat or solvents. The hemorrhage from the point of puncture was controlled and the wound closed in layers. The animals were permitted to recover and live for varying periods, from 6 hours up to 30 days. They were then exsanguinated by opening the femoral arteries and veins without the use of anesthetics. Each heart was examined grossly and microscopically after hardening in formaldehyde for several days. A description was made of the exterior and of surfaces made by cutting the heart from apex to base into transverse segments several millimeters thick. Microscopic study of the changes included the examination of sections made of frozen tissue stained with Sudan III, and of others stained with hematoxylin and eosin, celloidin preparations stained by Marchi, and other methods.

Gross Alterations

The immediate effects varied with the amount of oil injected. If more than 1.5 to 2 cc. enters the artery the heart becomes purplish and dilates, its rate first increases then diminishes, its rhythm becomes irregular, ventricular fibrillation ensues and finally the ventricles stop before the auricles. If the amount of oil entering the artery is less than 2 cc. an ill-defined area of varying size about the site of injection becomes purplish; the heart may dilate somewhat but its rate and rhythm are not appreciably changed. When the needle is withdrawn there is usually some subepicardial hemorrhage about the puncture.

The earliest change was noted at 6 hours and consisted of a deposit of fibrin on the epicardium about the injected artery and a mottling of dark to paler red-brown; beneath this the lesion continued in the wall as an irregularly but fairly well-defined region. Later, at 12 hours, the fibrinous deposit became thicker; the dark red-brown areas more sharply defined and the muscle somewhat granular and brown to pale yellow. Gradually, the dark brown places were surrounded by pale yellow-brown borders more sharply demarcated and still more granular and friable (24 hours). At 51 hours the centers of the dark brown areas were rusty brown. De-

marcation of the dark regions alternated with opaque yellow-white granular spots was increased by the appearance of pale bluish gray borders after 6 days. By the end of 10 days all these changes were well advanced. In such a heart the pericardium is adherent to the epicardium over the front upper surface about the site of injection (see Fig. 1). On surfaces made by cutting the bluish gray borders about the dark brown regions, the lines traversing these areas are depressed, translucent and broader. The central portions of these dark areas are yellow-brown. Yellow-white opaque areas are surrounded by similar borders and traversed by similar lines.

As time goes on the dark and the white areas are more and more replaced by this invading bluish gray tissue and small depressions and furrows with bluish gray translucent floors are evident externally. Later (20 days) the altered regions are converted into irregular but sharply circumscribed, soft, depressed brown areas on the surfaces made by cutting, and finally (30 days) the ventricular wall in such regions becomes considerably thinned. In a 30 day heart the pericardium is firmly adherent to the epicardium on the front surface (see Fig. 2) in several places. There are several irregular depressions on the front lower surface up to 1.5 mm. deep with bluish gray translucent floors. On surfaces made by cutting into these depressions the left ventricular wall is 3 mm. thick. Elsewhere at its thinnest portion this wall is 9 mm. in thickness. The muscle in these thinned places is almost entirely replaced by soft, dark brown, depressed tissue. There are two small areas, elsewhere on the front wall, up to 1 mm. in diameter which are soft, dark brown and depressed with puckered margins.

Histological Examination

In the beginning (6 hours) there accumulate large numbers of polymorphonuclear leukocytes, lymphocytes and extravasated red blood corpuscles between the muscle fibers which are torn and separated by globules and irregular masses of fat. In the largest of such places these cells are enmeshed in a network of fibrin. Some of the muscle fibers about these lesions are already granular and hyaline with indistinct nuclei. A little later these changes become more extensive. Red blood cells, lymphocytes and polymorphonuclear leukocytes are diffusely scattered over entire sections in the inter-

stices of the muscle. Especially dense are these extravasations and accumulations beneath the endocardium. Most of the fat here is in the form of long, sausage-shaped emboli distending capillaries, following their tortuous course and filling the bifurcations with Y-shaped figures. Only where the cellular accumulation and blood extravasation are most dense have the muscle fibers become hyaline and granular with pale, indistinctly staining nuclei.

At 24 hours more of the muscle fibers become completely hyalinized or granular, increased in diameter, with nuclei which are either poorly stained and pyknotic, or disintegrated into small irregular bodies which stain deeply with basic dyes. In the fibrin adherent to the epicardium polymorphonuclear leukocytes and lymphocytes accumulate.

Gradually the altered muscle is removed and replaced by young fibrous tissue. For example, after 42 hours the granular and hyaline muscle fibers are distorted and fragmented. Closely apposed to the ends of these fibers are numerous small, multinuclear cells about 10 micromillimeters in diameter, containing up to six dark purple-staining nuclei with very pale staining cytoplasm. Among the accumulated cells, especially in the interstitium and about the capillaries, young fibroblasts with oval and spindle vesicular nuclei with numerous mitoses are present. Here and there is a nucleus in the altered muscle resembling the Anitschkow "myocyte" [10] with a slightly central or eccentric, dark staining body with fine chromatin lines radiating peripherally. Many capillaries are still distended by sausage-shaped emboli of fat.

Later on, at 51 hours, the extravasated red cells are crenated and pale, the disintegrated muscle further resorbed, the accumulated cells fewer and fibroblasts more numerous. About some of the fat masses there are very minute droplets of fat in the muscle fibers (Fig. 3) as Warthin noted: "In the Marchi preparations around the blackened masses of fat are numerous muscle fibers, the sarcoplasm of which contains a dense accumulation of fine, black granules and small globules, arranged in longitudinal lines. The striations of muscle fibers with most numerous granules are absent or very faint."

At 10 days, regions of degenerated muscle cells between sausage-shaped, tortuous fat emboli remain as islets in broad bands of dense, young fibrous tissue. The resorption of the fat goes on apace (see Fig. 4). This gradual removal of the shrunken hyalinized fragments

of muscle and of fat continues (16 days) until at 20 days little else is left in the altered places except dense, vascular fibrous tissue containing free globules of fat. Oval-shaped phagocytes filled with coarse globules of fat, and others with a fine granular yellow-brown pigment, are numerous. After 30 days the fibrous tissue, still denser, almost completely replaces the muscle in one region from endocardium to epicardium (Fig. 5). But even after 30 days fat globules are still present in the fibrous tissue (Fig. 6).

Discussion

Unchanged oil from canine adipose tissue was regarded as perhaps less objectionable than an oil quite foreign to dogs. The hemorrhage, infiltration with leukocytes, necrosis, appearance of fat in the muscle fibers, gross alterations of color, disappearance of necrotic tissue and cicatrization have all been described by others.[10, 11] The differences we observed in these infarcts produced by canine fat were, in some particulars, related to the physical nature of the material used. The oil spread out in the capillaries in a manner similar to the distribution Wearn [12] obtained of material he injected to study the capillary bed of the myocardium. The small size of the infarcted regions we produced in dogs' hearts with oil may be due to the liberal anastomosis Wearn demonstrated, and to the absence in the preparations we studied of vessels larger than capillaries plugged by the oil. Apparently infarction followed permanent blocking with oil of all the capillaries in certain limited portions of the myocardium, rather than occlusion of some large or medium-sized artery, as takes place naturally with coronary thrombosis and embolism, and which has usually resulted in most of the occlusion experimentally produced. This contrast between these oil infarcts and the usual type in which vessel blockade is confined to the periphery is particularly noteworthy, notwithstanding the significance of the dissimilarity is possibly limited to infarcts produced experimentally. The depressions found on the outside of the hearts of dogs 16 to 30 days after injection of the oil suggest that the capillaries where the oil lodged belong to the perpendicular branches of the subepicardial division of the arteries described by Whitten.[13]

We were surprised to find some of the oil still present after 30 days in the cicatrices. In preparations examined 6 days after injection

there were minute drops of oil in large phagocytic cells; evidently it is disposed of very slowly. About some, but not about all of the emboli of fat, the adjacent muscle fibers were fatty, as noted by Warthin. We are unable either to confirm or refute his opinion that the fat in such fibers was embolic and had accumulated in the muscle after passing through the capillary walls. Some of the droplets were larger than those found in the usual fatty degeneration of the myocardium, the "herring-bone" or "tabby-cat" fatty change, for example. It may not be proper to assume that scars such as we studied would be widely disseminated throughout the myocardium if the oil had passed out through both coronary arteries so as to attain a wide distribution in the heart muscle. On the other hand, Vance [14] has reported minute streaky or flame-like hemorrhages several millimeters long scattered throughout the human myocardium from fat embolism due to automobile injuries. He also states that permanent scars due to "this process" have not been described. Perhaps pulsations of the human heart so aid in removal of fat emboli that minute focal scars do not follow, or possibly fat embolism should be added to the long list of causes now held responsible for disseminated minute scars throughout the myocardium, the so-called interstitial myocarditis.

A third possibility is that death occurs too soon for such scattered scars to develop throughout the myocardium, that with fat embolism sufficient to produce these focal lesions in the heart, other organs, and especially the brain, are also involved and patients so afflicted die at the height of systemic generalized fat embolism.

REFERENCES

1. Lehman, E. P., and Moore, R. M. Fat embolism; including experimental production without trauma. *Arch. Surg.*, 1927, **14**, 621–662.

2. Graham, G. S. Fat embolism; report of a case and of experiments on animals. *J. Med. Res.*, 1907, **16**, 459–482.

   Gröndahl, N. B. Untersuchungen über Fettembolie. *Deutsche Ztschr. f. Chir.*, 1911, **111**, 56–124.

   Warthin, A. S. Traumatic lipaemia and fatty embolism. *Internat. Clin.*, 1913, **4**, Series 23, 171–227.

   Landois, F. Die Fettembolie. *Deutsche med. Wchnschr.*, 1926, **52**, 283–285.

3. Gold, E., and Löffler, E. Experimentelle Untersuchungen zur Pathogenese der Fettembolie. *Ztschr. f. d. ges. exper. Med.*, 1923, **38**, 153–173.

4. Porter, W. T. Shock from fat embolism of vasomotor center. *Am. J. Physiol.*, 1924–25, **71**, 277–315.

5. Paul, F., and Windholz, F. Experimentelle Studien über die Fettembolie und den durch sie verursachten Tod. *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1924–25, **38**, 614–651.

6. Weimann, W. Über die Hirnveränderungen bei cerebraler Fettembolie. *Deutsche Ztschr. f. d. ges. gerichtl. Med.*, 1929, **13**, 95–103.

7. Vanucci, D., and Franceschini, P. Sull' embolia grassosa (Richerche experimentali). *Arch. ital. di chir.*, 1926, **16**, 585–681.

8. Ziemke, E. Zum akuten fettembolischen Tode nach leichten Erschütterungen. *Deutsche Ztschr. f. d. ges. gerichtl. Med.*, 1922, **1**, 193–203.

9. Rukstinat, G. Experimental air embolism of the coronary arteries. *J. A. M. A.*, 1931, **96**, 26–28.

10. Karsner, H. T., and Dwyer, J. E., Jr. Studies in infarction IV. Experimental bland infarction of the myocardium, myocardial regeneration and cicatrization. *J. Med. Res.*, 1916, **34**, 21–39.

11. Mönckeberg, J. G. Die Erkrankungen des Myokards und des spezifischen Muskelsystems. Handbuch die speziellen pathologischen Anatomie und Histologie, Henke, F., and Lubarsch, O. J. Springer, Berlin, 1924, **2**, 383–398.

12. Wearn, J. T. The extent of the capillary bed of the heart. *J. Exper. Med.*, 1928, **47**, 273–292.

13. Whitten, M. B. The relation of the distribution and structure of the coronary arteries to myocardial infarction. *Arch. Int. Med.*, 1930, **45**, 383–400.

14. Vance, B. M. Fatal fat embolism. *Arch. Path.*, 1919, **7**, 554–558. (Proc. New York Path. Soc. Dec. 15, 1928.)
The significance of fat embolism. *Arch. Surg.*, 1931, **23**, 426–465.

DESCRIPTION OF PLATES

PLATE 9

FIG. 1. (10 days.) The region of infarction is easily followed from one to another in these segments of the ventricles. In the more apical it is darker than the rest of the myocardium; toward the base beginning cicatrization has made it paler. Part of the pericardial sac with a little of the left lung is bound fast by fibrous adhesions to a portion of the periphery of the first four segments.

FIG. 2. (30 days.) Considerable of the outside of the heart is covered by adherent pericardium. Near the apex is one deep dent. Above and to the left several smaller depressions are near the right border. These are all infarcts partly cicatrized.

1

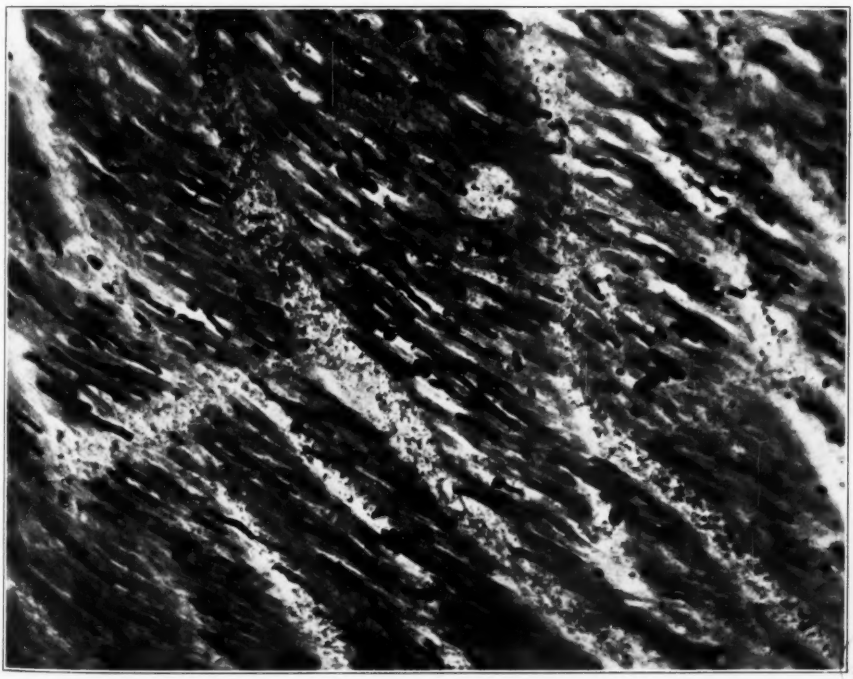
2

PLATE 10

FIG. 3. (51 hours.) The large pear-shaped region is filled with blood in a meshwork of fibrin. The injected oil is black as are also fine droplets of fat in the muscle fibers. Marchi and Bismark brown.

FIG. 4. (10 days.) Extensive occlusion of capillaries by sausage-shaped emboli of dog oil. The torn places, most of them extending up and to the left, are filled with erythrocytes and among these are minute drops of fat in phagocytes. Marchi and Bismark brown.

3



4

Szurek and Czaja Experimental Fat Embolism of Heart

PLATE 11

FIG. 5. (30 days.) A low power illustration of one of the infarcts with myocardium replaced by highly vascular granulation tissue. The wall of the left ventricle here was only 3 mm. thick. Hematoxylin and eosin.

FIG. 6. (30 days.) Large masses of dog oil in granulation tissue well advanced toward cicatrization.

5

6

BODY LENGTH AND ORGAN WEIGHTS OF INFANTS AND CHILDREN *

A STUDY OF THE BODY LENGTH AND NORMAL WEIGHTS OF THE MORE IMPORTANT VITAL ORGANS OF THE BODY BETWEEN BIRTH AND TWELVE YEARS OF AGE

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Owing to the meager data regarding weights of normal organs of infants and children, it seemed advisable to make use of our records for this purpose. Our intention was only to do sufficient work to check up on published tables. Impetus carried us beyond this point and led us to examine all our available data and then to prepare for our own use tables and graphs for handy reference. These we have used to our own satisfaction for three years and we now publish them for what they may be worth.

The graphs are self-explanatory. After due consideration we decided not to elaborate upon them, believing that the information as presented is amenable to such mathematical treatment as may seem desirable by persons interested. However, the data employed may be obtained from us, as well as those employed for graphs omitted from this article, in which the weights of the same organs have been plotted according to body length. For the benefit of those having use for the data included in the graphs, we include information about the methods of selection of material.

At the Children's and Infants' Hospitals 2287 autopsy records were examined, of which 1043, or 45.6 per cent, were selected. Each selected autopsy contributed a normal weight of at least one vital organ. Similarly, 7194 autopsy records were examined at the Boston City Hospital, of which 155 or 2.15 per cent, between the ages of 6 and 12 years were selected. These latter autopsies supplemented the data from the Children's and Infants' Hospitals, making a total of 1198. Upon these the substance of this study is based.

* Received for publication May 31, 1932.

The following is a tabular summary of the records examined:

Hospital	Age	No. records examined	Years cases selected	No. selected	Per cent selected	Total per cent selected
C. H. and I. H.	Birth to 6 yrs.	2287	1914–1929	1043	45.6	87
B. C. H.	6 to 12 yrs.	7194	1896–1929	155	2.15	13
Total		9481		1198	47.75	100

The information gained from each selected autopsy record was tabulated in the following manner:

Age period	Autopsy No.	Age	Body length	Heart	Lungs		Spleen	Liver	Kidneys		Brain
					Right	Left			Right	Left	
6 mos.	26–130	6 mos.	*cm.* 58	*gm.* 34	*gm.* 45	*gm.* 32	*gm.* 16	*gm.* 225	*gm.* 26	*gm.* 23	Restricted

Similarly the body length in centimeters and the weight of the various organs in grams were catalogued under the particular age period to which the case belonged. If an organ was recorded as diseased or otherwise abnormal, it was so marked and later rejected. When no demonstrable pathological changes were described, the weight of the organ was accepted as normal. Prematurity was sufficient reason for rejection.

The selected cases were separated according to age into thirty-two periods, *e. g.*, birth to 3 days (inclusive), 3 to 7 days, 1 to 3 weeks and so on (Table I). The average weight in grams of each organ and the average body length in centimeters for each age period were then computed. The figures are given in Table I. An attempt was then made to show graphically how the various figures for each organ at every age period were distributed above and below the computed arithmetical mean. Thus, the upper and lower limits of normal were ascertained. For each age period along the growth graph of each organ there was charted the number of organ weights which fell within 5 grams (in the lungs, 10 grams; in the liver and brain, 50 grams, and so on) of each other, above and below the mean weight.

A similar graphic chart was constructed for the body length (Table II). Each organ weight chart presents two graphs; a lower general growth graph between birth and 12 years, and a detailed upper

graph between birth and 2 years. Both graphs give the mean weight of the organ at various age periods, with a perpendicular scale showing the number and distribution of the individual weights which yielded the arithmetical mean. The upper and lower weight limits as depicted on the perpendicular scales are arbitrary and not actual values within which actual weights are grouped. A sample working sheet of the heart at the age period of 1 to 3 weeks is shown as follows:

Grams	31	26	21	16	11	6
Instances	2	12	21	7	1	

Here the upper line of figures represents the arbitrary weight of the heart distributed at 5 gram intervals from 31 down to 6 grams. The lower line represents the number of individual weights which fall within each 5 gram interval. The actual weights within each interval were represented on the working sheets as follows:

Grams	Individual Weights	Total Weights
31–26	30, 28	2
26–21	21, 22, 25, 25, 25, 23, 23, 23, 25, 25, 23, 22	12
21–16	20, 20, 20, 20, 20, 20, 18, 18, 17, 19, 18, 18, 18, 18, 18, 18, 18, 17, 17, 17, 17	21
16–11	12, 14, 14, 14, 15, 15, 15	7
11– 6	10	1
	Total No. of Weights	43
	Total Weight	835 grams
	Mean Weight	19 grams

The lowest arbitrary figure in this sample sheet is 6 grams, although the lowest actual weight is 10 grams, which falls in the interval between 6 and 11 grams. Likewise there are seven cases between 11 and 16 grams, and so on. A similar system was used in the charts representing body length and the weights of the other organs, except that in the lungs cases were grouped within intervals of 10 grams, and in the liver and brain 50 grams.

On a single chart (Chart 10) the general growth graphs of all organs and body length are represented. The ordinates and abscissae are relabeled so that all graphs could be included. The graphs for the left lung and the left kidney are omitted because they correspond in general to the graphs for the right lung and kidney. No figures are available for the normal weights of lungs between 11 and 12 years of age.

Because there were such wide variations, as shown in the charts, above and below the calculated arithmetical mean, another group of charts was prepared representing a correlation between organ weight and body length in order to show the weights of organs at any given body length. The same method used above in calculating the organ weight at each age period was employed. The weights were grouped within intervals of 5 centimeters from 45 to 140 centimeters of body length. Having calculated the mean weight of each organ at various height levels, charts were drawn and the individual weights were grouped as before on an arbitrary vertical scale above and below the calculated mean weight of the organ. A similar, but slightly less marked variation in the upper and lower limits of normal occurred in the second set of charts (not reproduced here).

A comparison of our figures with those of other authors is given below:

WEIGHT OF HEART

Age Period	*Boyd*[1]	*Authors'*
Birth	20.6 gm.	17 gm.
1½ yrs.	44.5 "	52 "
3 yrs.	60.2 "	59 "
5½ yrs.	72.8 "	85 "
10½ yrs.	122.6 "	122 "

WEIGHT OF BRAIN

Age Period	*Feer*[2]	*Authors'*
Birth	370 gm.	335 gm.
2 mo.	460 "	489 "
4–6 mo.	600 "	644 "
11–12 mo.	850 "	925 "
2 yrs.	970 "	1064 "
3 "	1100 "	1141 "
4 "	1190 "	1191 "

NORMAL WEIGHTS OF ORGANS OF NEW-BORN

Organ	*Craig*[3]	*Authors'*
Brain	385 gm.	335 gm.
Heart	24 "	17 "
Kidneys	23 "	right 13 "
Liver	118 "	78 "
Lungs	58 "	right 21 "
Spleen	8 "	8 "

BODY LENGTH

Age Period	*Feer*	*Holt & Howland*[4]	*Authors'*	*Age Period*	*Feer*	*Holt & Howland*[4]	*Authors'*
Birth	50 cm.	52.0 cm.	49 cm.	6 yrs.	109 cm.	... cm.	109 cm.
6 mo.	.. "	67.4 "	62 "	7 "	115 "	... "	113 "
12 mo.	75 "	75.0 "	73 "	8 "	120 "	... "	119 "
18 mo.	.. "	80.0 "	78 "	9 "	125 "	... "	125 "
2 yrs.	85 "	85.1 "	84 "	10 "	130 "	... "	130 "
3 "	93 "	94.0 "	88 "	11 "	135 "	... "	135 "
4 "	99 "	... "	99 "	12 "	140 "	... "	139 "
5 "	104 "	... "	106 "				

REFERENCES

1. Boyd. Weight of heart at different ages. In Pediatrics, edited by Isaac A. Abt. W. B. Saunders Co., Philadelphia, 1923, 1, 946.
2. Feer, E. Textbook of Pediatrics. Translated by Sedgwick, J. P., and Scherer, C. A. J. P. Lippincott Co., Philadelphia, 1922, 1.
3. Craig, C. F. Laboratory Methods of the U. S. Army. Lea and Febiger, Philadelphia, 1929, Ed. 3.
4. Holt, L. E., and Howland J. The Diseases of Infancy and Childhood. D. Appleton and Co., N. Y., 1926, Ed. 9.

TABLE I. ORGAN WEIGHTS

Children's and Infants' Hospital Series

Supplemented Between 6 to 12 Years by Data from the Boston City Hospital

Age	Body length	Heart	Lungs		Spleen	Liver	Kidneys		Brain
			Right	Left			Right	Left	
	cm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Birth–3 days	49	17	21	18	8	78	13	14	335
3–7 "	49	18	24	22	9	96	14	14	358
1–3 wks.	52	19	29	26	10	123	15	15	382
3–5 "	52	20	31	27	12	127	16	16	413
5–7 "	53	21	32	28	13	133	19	18	422
7–9 "	55	23	32	29	13	136	19	18	489
9–3 mos.	56	23	35	30	14	140	20	19	516
4 "	59	27	37	33	16	160	22	21	540
5 "	61	29	38	35	16	188	25	25	644
6 "	62	31	42	39	17	200	26	25	660
7 "	65	34	49	41	19	227	30	30	691
8 "	65	37	52	45	20	254	31	30	714
9 "	67	37	53	47	20	260	31	30	750
10 "	69	39	54	51	22	274	32	31	809
11 "	70	40	59	53	25	277	34	33	852
12 "	73	44	64	57	26	288	36	35	925
14 "	74	45	66	60	26	304	36	35	944
16 "	77	48	72	64	28	331	39	39	1010
18 "	78	52	72	65	30	345	40	43	1042
20 "	79	56	83	74	30	370	43	44	1050
22 "	82	56	80	75	33	380	44	44	1059
24 "	84	56	88	76	33	394	47	46	1064
3 yrs.	88	59	89	77	37	418	48	49	1141
4 "	99	73	90	85	39	516	58	56	1191
5 "	106	85	107	104	47	596	65	64	1237
6 "	109	94	121	122	58	642	68	67	1243
7 "	113	100	130	123	66	680	69	70	1263
8 "	119	110	150	140	69	736	74	75	1273
9 "	125	115	174	152	73	756	82	83	1275
10 "	130	116	177	166	85	852	92	95	1290
11 "	135	122	201	190	87	909	94	95	1320
12 "	139	124	...	...	93	936	95	96	1351

TABLE II. BODY LENGTH

Children's and Infants' Hospitals Series
Supplemented Between 6 to 12 Years by Data from the Boston City Hospital

Age	Body length in cm.	No. cases	Age	Body length in cm.	No. cases
Birth–3 days	49	15	14 mos.	74	30
3–7 "	49	15	16 "	77	26
1–3 wks.	52	29	18 "	78	22
3–5 "	52	30	20 "	79	21
5–7 "	53	16	22 "	82	11
7–9 "	55	40	24 "	84	20
9–3 mos.	56	53	3 yrs.	88	36
4 "	59	36	4 "	99	14
5 "	61	41	5 "	106	16
6 "	62	43	6 "	109	36
7 "	65	35	7 "	113	41
8 "	65	29	8 "	119	21
9 "	67	18	9 "	125	27
10 "	69	20	10 "	130	20
11 "	70	20	11 "	135	17
12 "	73	11	12 "	139	16

In all charts of weights (Charts 2 to 9 inclusive) the column of figures on the left refers to grams, that on the right in Chart 10, to centimeters.

In Chart 1, the column on the left refers to centimeters. In all charts, the upper line of horizontal figures is the age scale for the upper graph, the lower line for the lower graph.

Body Length

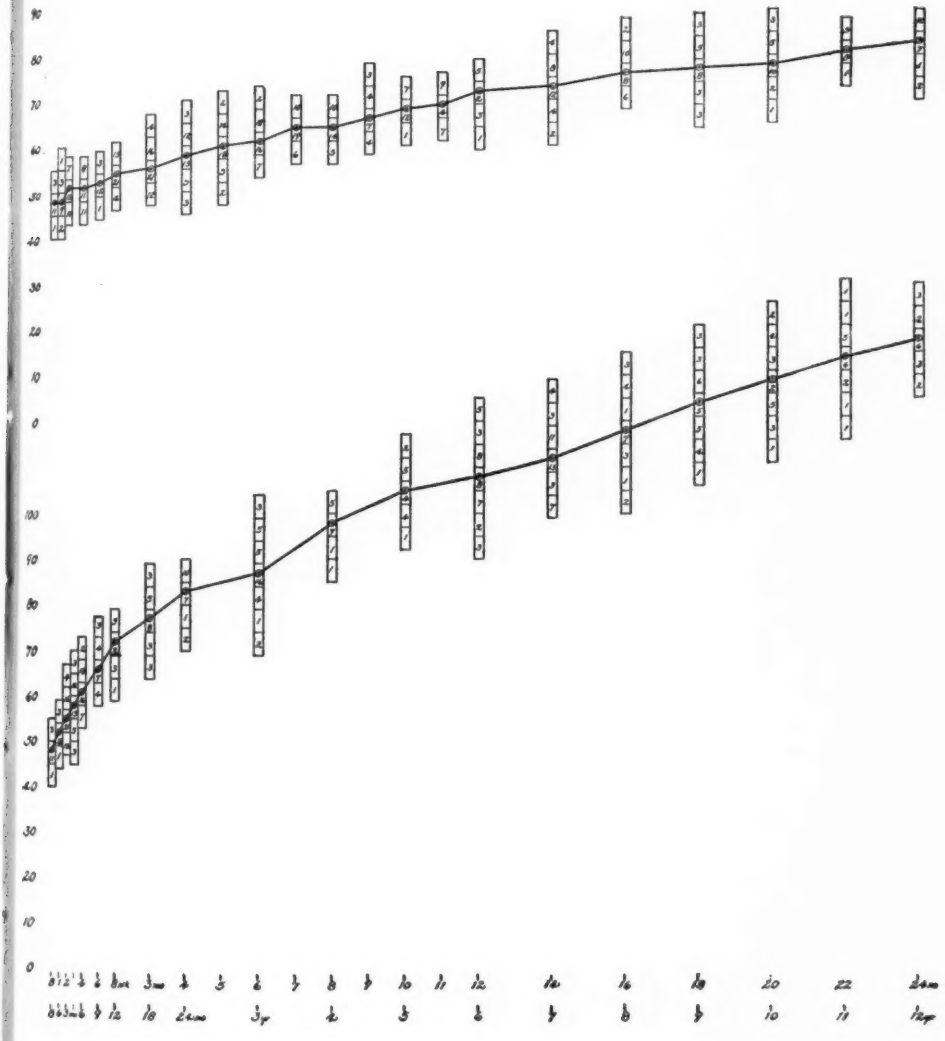

Chart 1

HEART

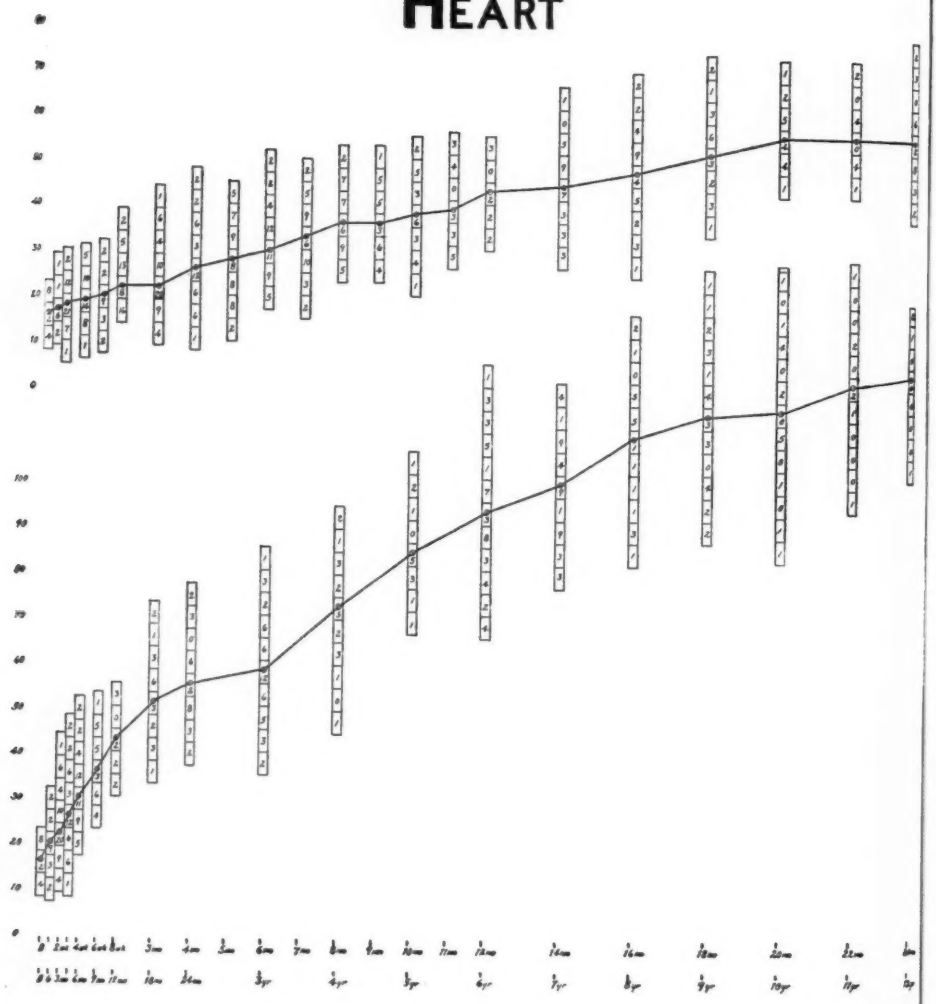

CHART 2

RIGHT LUNG

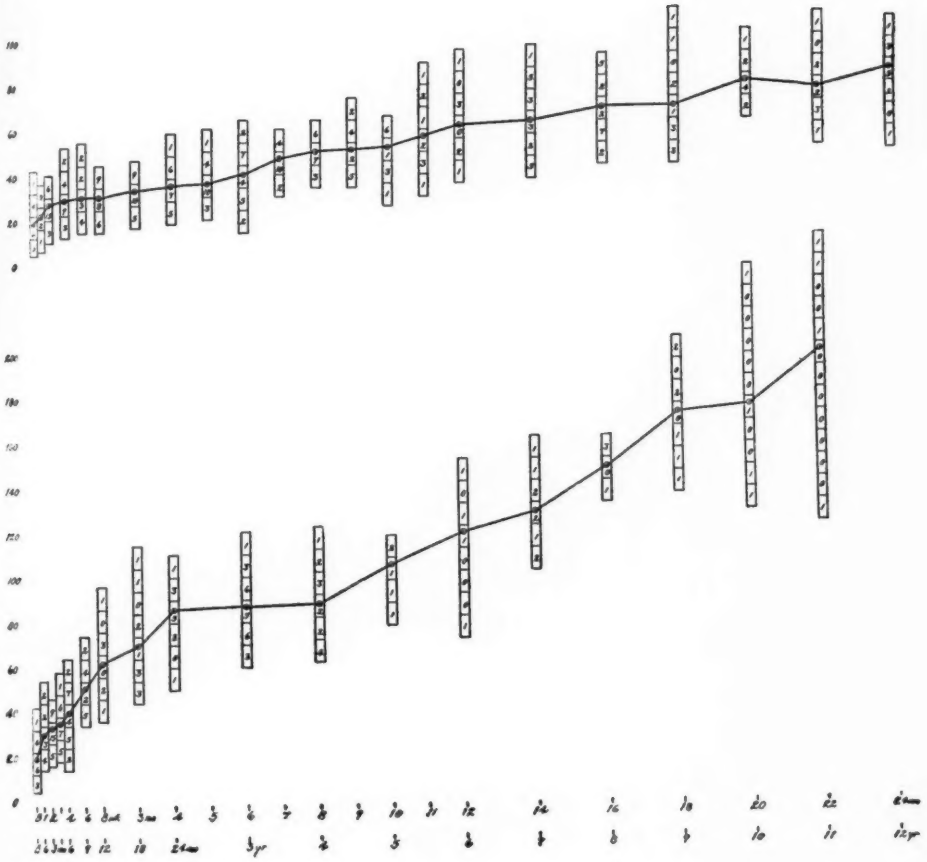

CHART 3

LEFT LUNG

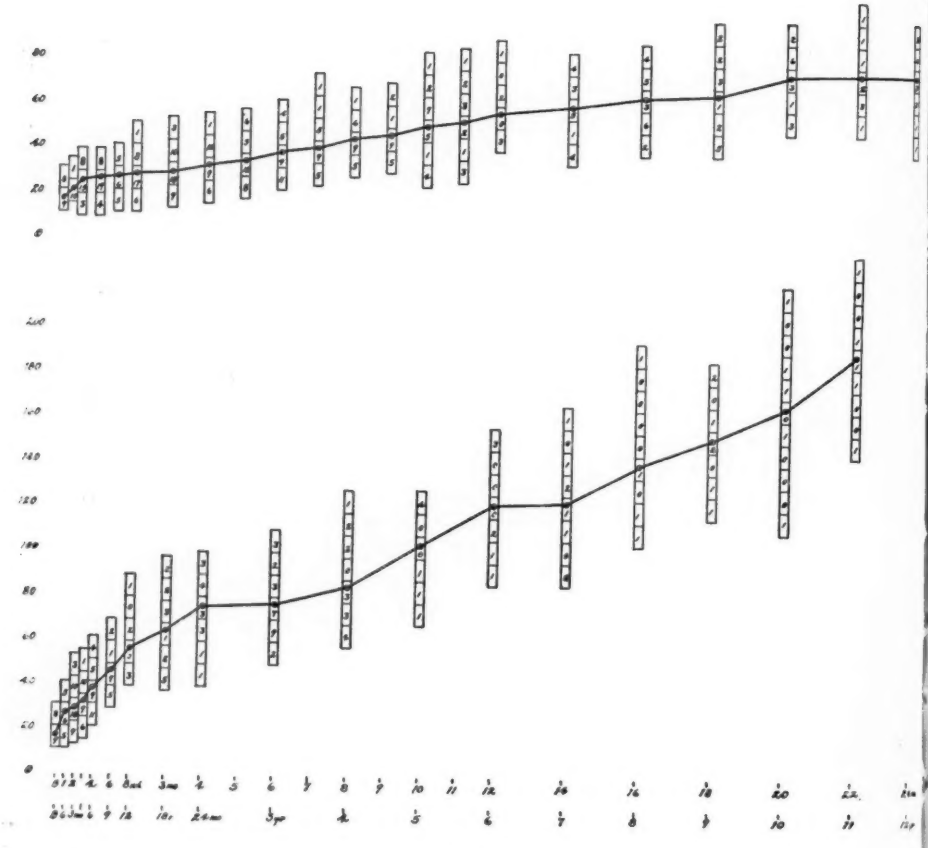

CHART 4

SPLEEN

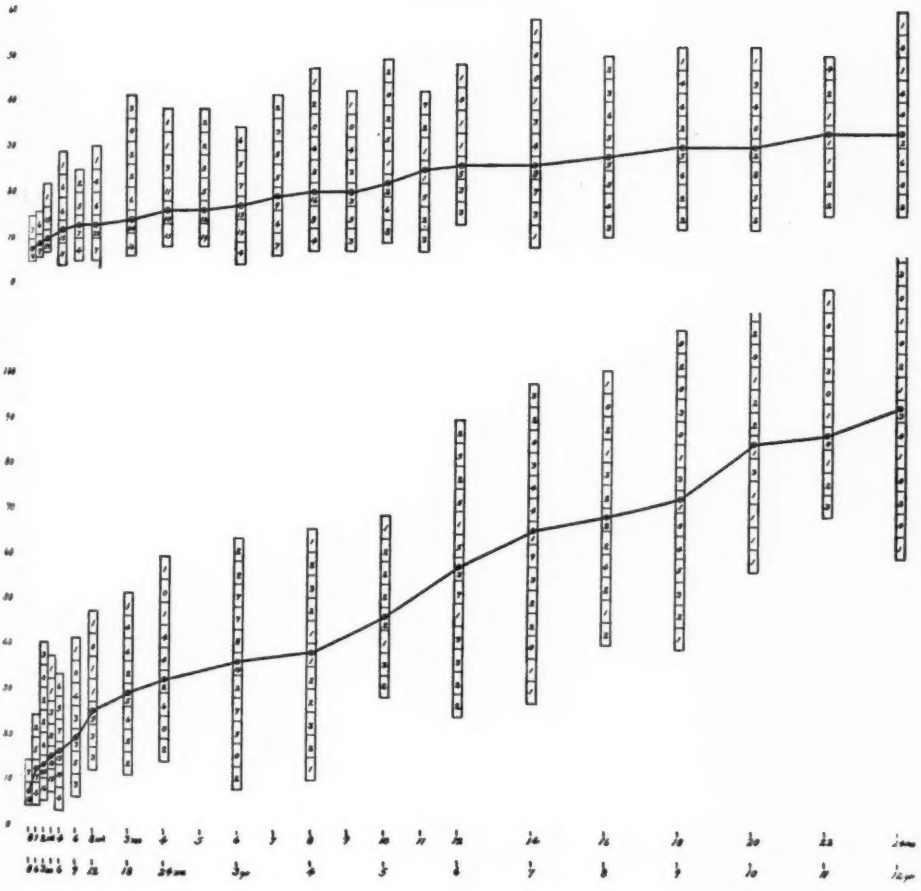

CHART 5

LIVER

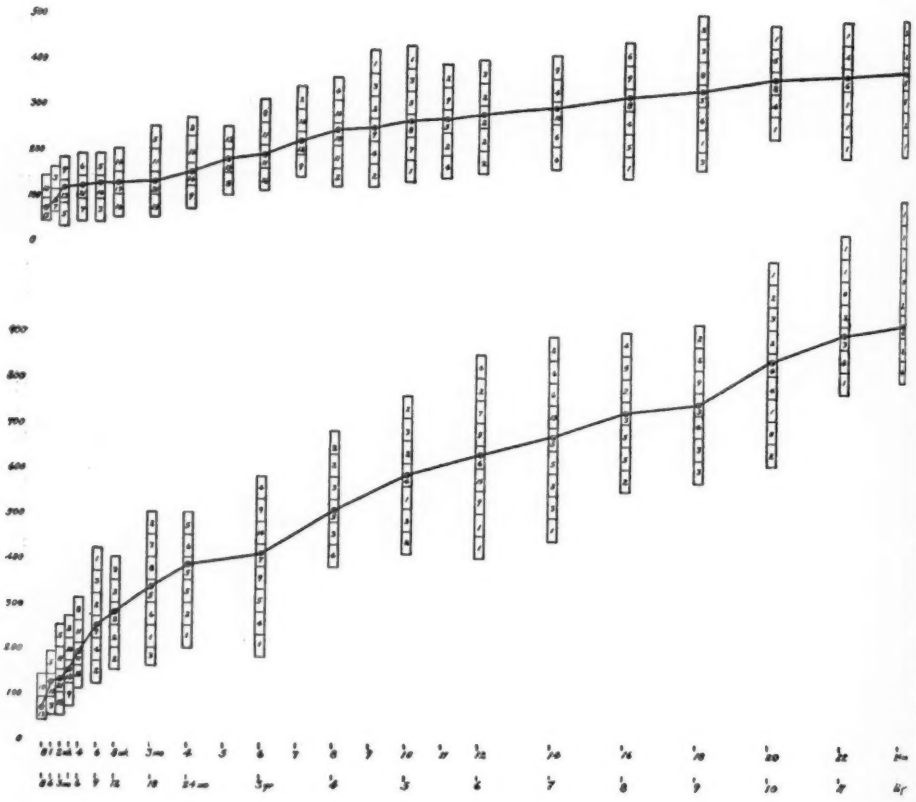

CHART 6

RIGHT KIDNEY

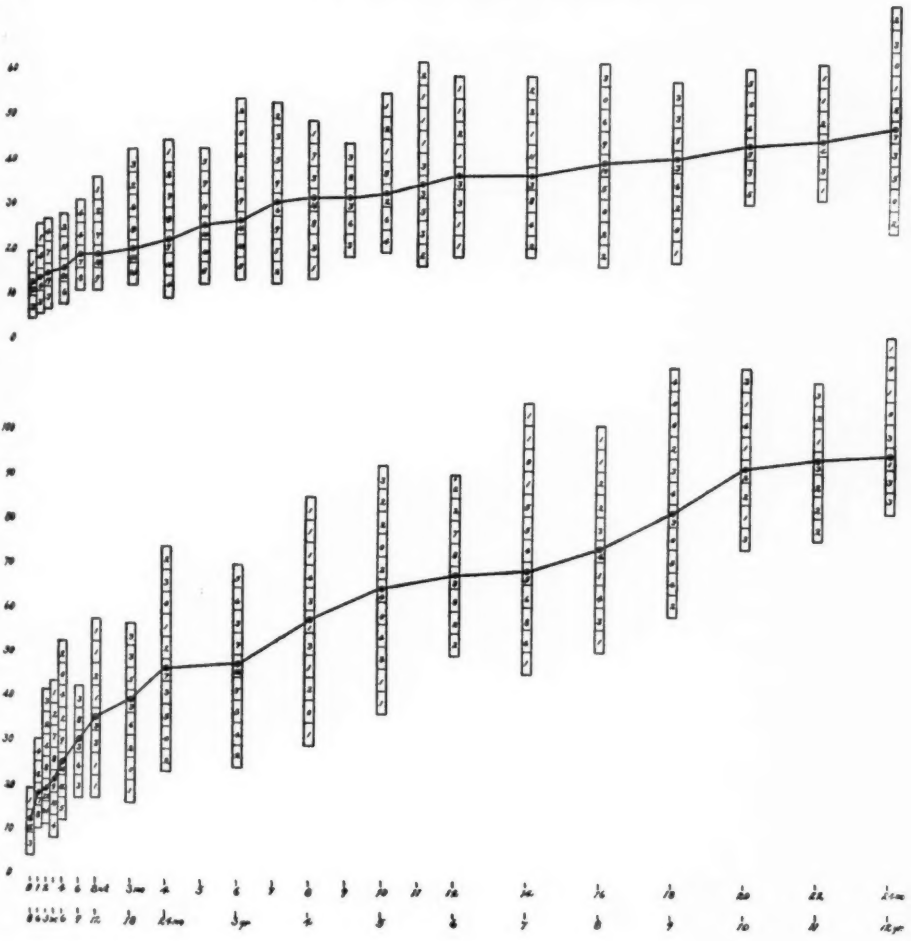

CHART 7

LEFT KIDNEY

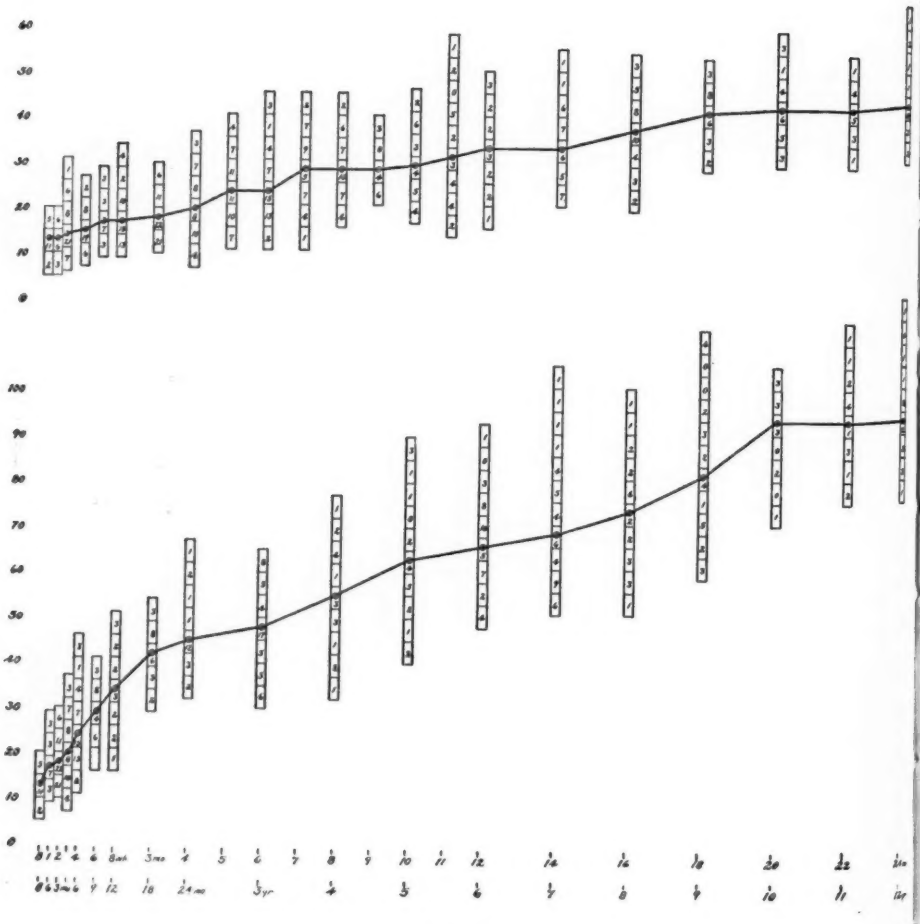

CHART 8

BRAIN

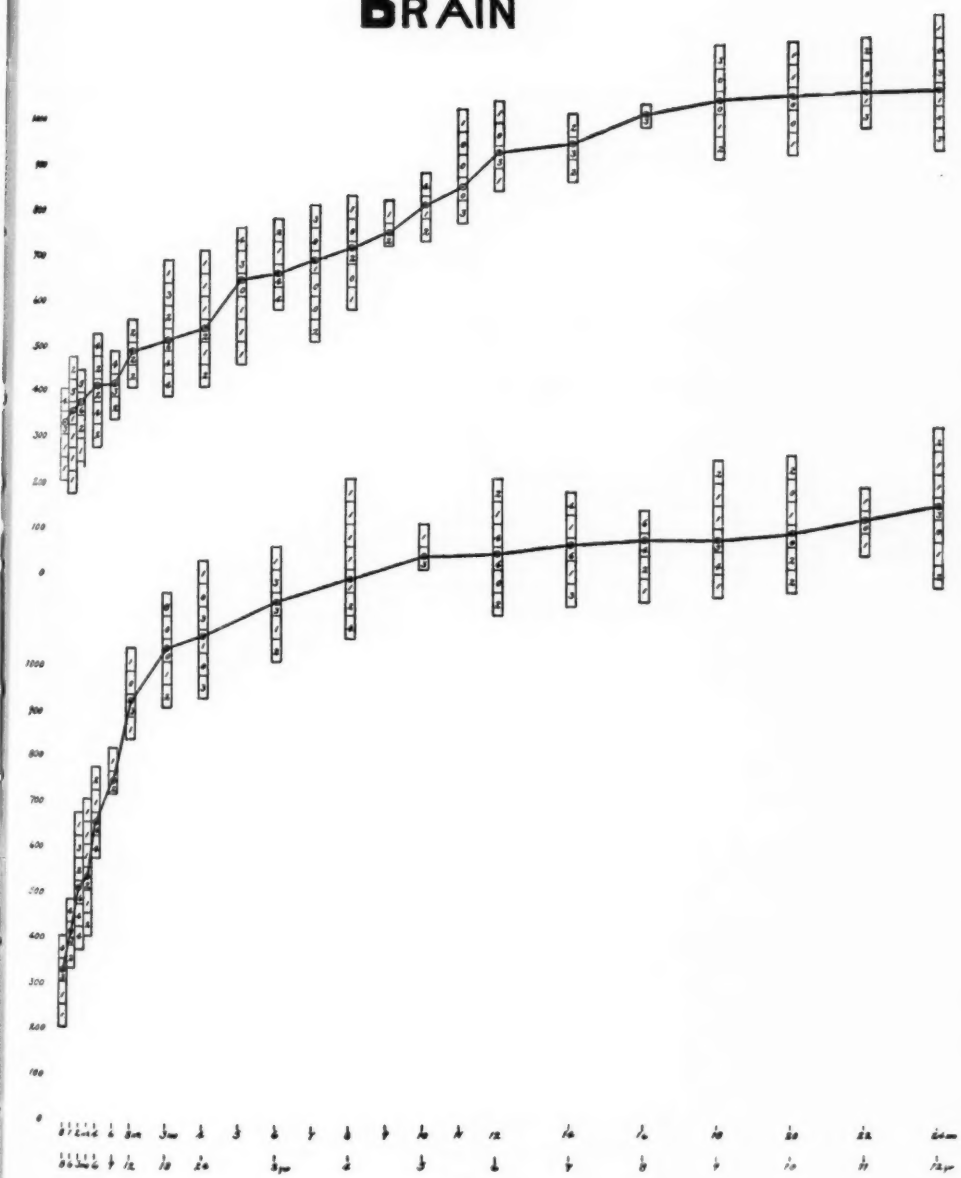

CHART 9

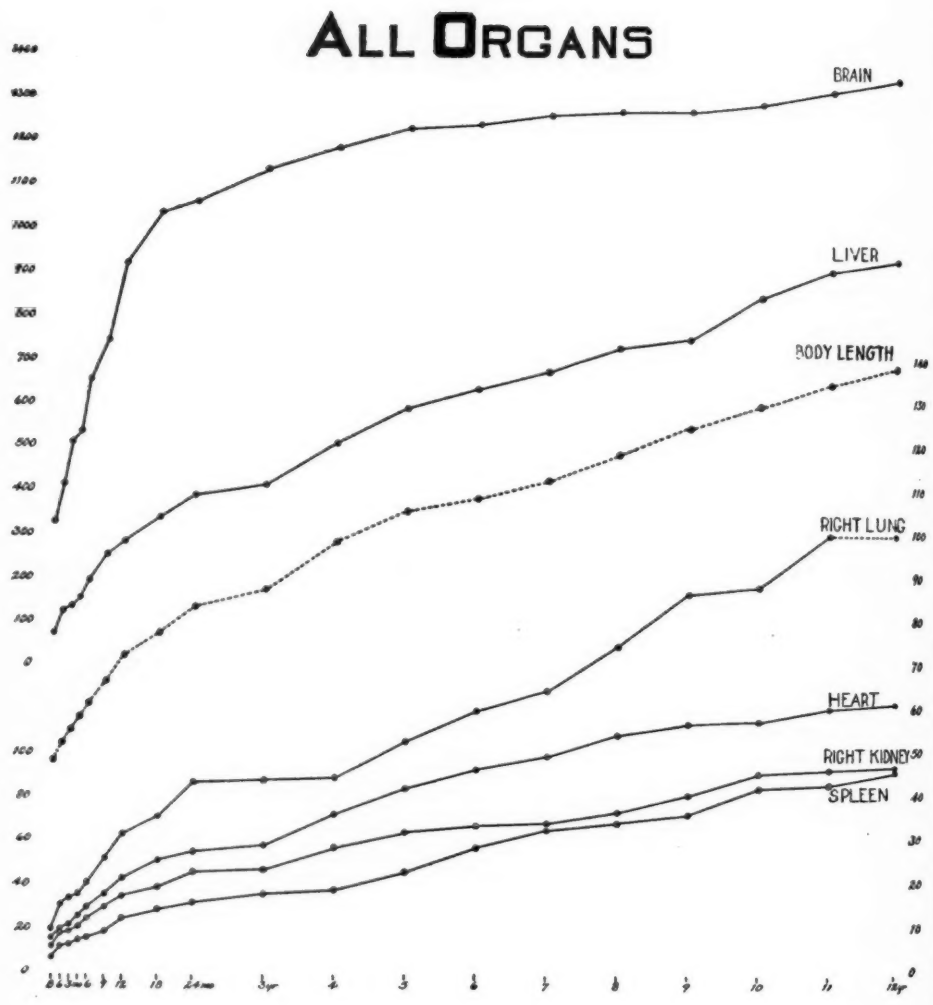
ALL ORGANS
BRAIN
LIVER
BODY LENGTH
RIGHT LUNG
HEART
RIGHT KIDNEY
SPLEEN

CHART 10

MULTIPLE LIVER ABSCESSES CAUSED BY A LEPTOTHRIX, WITH A REVIEW OF LEPTOTHRICAL INFECTION *

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Mycotic abscesses of the liver are quite uncommon, and of those encountered the etiological agent is usually an actinomyces.[1] As yet there are no cases on record of liver abscesses produced by a cladothrix or leptothrix. The case herewith reported is one in which multiple abscesses of the liver were found to be caused by a leptothrix which was successfully grown on artificial culture media.

The members of the leptothrix group of microörganisms have a very low pathogenicity, but have been grown on artificial culture media only rarely. According to Huntemüller [2] they are frequently found as saprophytes in the human mouth. Von Beust [3] found leptothrices regularly in gingivitis and stomatitis, but their significance is uncertain. Mendel [4] isolated an anaerobic leptothrix from a case of alveolar pyorrhea and found it pathogenic for animals. Infection of the lachrymal canals by leptothrices with production of Parinaud's conjunctivitis is fairly common, and infection of the pharynx with these microörganisms is even more so; in the latter case a pseudomembrane closely resembling that seen in diphtheria is formed which may be very resistant to treatment, lasting for months without causing much inconvenience.[2]

In 1896 Flexner [5] reported the isolation of a pathogenic leptothrix from a rabbit which died five days after parturition. Acute pleuritis, pericarditis and pneumonia were associated with acute endometritis, all caused by the same organism. Cultures were obtained only by the use of fresh tissue, fetal tissue giving the best results. The organism was highly pathogenic for rabbits. A summary of the literature relating to cladothrix and leptothrix infections concludes his article.

Follet and Sacquépée [6] in 1902 reported the isolation of a leptothrix from the blood stream and from the vesicles of a patient with an atypical zona. The spinal fluid was normal. Their organism

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seems to have been a cladothrix, for they saw false branching occasionally.

In 1901 Blumer and MacFarlane [7] reported a series of sixteen cases of noma following an epidemic of measles in an orphan asylum. Nine cases were examined bacteriologically, and in all a leptothrix was found. In early cases few other organisms were present, but later secondary invaders were abundant. The organism stained poorly by Gram's method but did not decolorize. The leptothrix could not be cultured.

In 1905 Mathews [8] reported a case of localized empyema due to a leptothrix. The organism appeared as free filaments, and more abundantly in colonies forming small yellowish granules. The filaments varied in length, stained readily but unevenly, showed irregular thickening, and their ends were often slightly swollen. These ends stained more deeply and tended to be Gram-positive, while the rest of the filament was Gram-negative. Cultures failed to grow and guinea pig inoculation with pus was fruitless.

In 1914 Schottmüller [9] isolated an organism from the blood of a woman who had been bitten by an African squirrel and subsequently developed pustules all over the body. Smears of the pus showed bacillary forms. Blood agar plates smeared with pus showed no growth grossly, but stained smears showed numerous sinuous filaments. Blood culture also showed no gross growth, but smears showed short filaments. Subcultures of these cultures showed no growth. Schottmüller calls this organism a streptothrix instead of a leptothrix because of "die schön geschwungene und gewundene Wuchsform in der Eiterkultur," although no branching was seen.

Deussing [10] in 1920 reported a case of leptothrix angina accompanied by infection with a diplococcus and complicated by glomerulonephritis. The nephritis he attributed to the coccus, but Huntemüller feels that it may have been due to the leptothrix. Since the patient recovered this question must remain unsettled. The occurrence of nephritis is of interest because of its presence in a case reported by Roedelius and Brack [11] in 1926. Their case was one of leptothrix mycosis of the tongue. The tongue became swollen and a small area of gangrene appeared. Incision for drainage was employed but no pus was found; a piece of tissue was excised for section and culture. Following operation a leptothrix stomatitis appeared, and later an acute glomerulonephritis was noted. Sections showed

many filamentous organisms in the tissue. Attempts to culture the organism failed.

In 1921 von Meyenburg [12] saw a case in which an extensive ulcerative process in the stomach was associated with the presence of many small abscesses in the liver. Sections of the stomach showed filamentous organisms in the mucosa and submucosa. The liver showed two apparently different processes, small abscesses with central masses of streptococci, and small areas of necrosis with secondary leukocytic invasion and a few scattered cocci. A few filaments were seen in the latter lesions, but not in the abscesses. In one section masses of leptothrices were seen loosely filling two branches of the portal vein. Cultures of the abscesses contained streptococci only.

Shortly thereafter Lang [13] reported a case of liver infarction associated with a chronic duodenal ulcer. There were many areas varying in size, club-shaped or rounded and irregular, of gray-green color, visible through the capsule and for the most part sharply circumscribed. Well defined zones of leukocytic infiltration about these areas were not seen, although a few foci of leukocytic infiltration were found. Within the bile passages at the edges of the affected regions were accumulations of long unbranched filaments and coccoidal bodies; both stained deeply with hematoxylin, but were Gram-negative. The nature of the coccoidal bodies was not clear, but they seemed to be growth forms of the leptothrix rather than staphylococci. The affected bile ducts were widened and their walls thickened. Even more massive clumps of filaments, and occasionally cocci, were seen in the portal veins, which also were for the most part greatly enlarged. The veins and some of the adjacent hepatic arteries were thrombosed, and contained masses of leptothrices. Frequently extension of filaments out into the sinusoids of adjacent and usually necrotic lobules was seen. Toxic action of the organism upon the tissues was not apparent, and definite abscess formation was lacking, the organisms acting purely mechanically by occlusion of vessels. Cultures of the organism were not obtained.

Galli-Valerio [14, 15] has repeatedly been able to isolate a leptothrix from cases of pleuritis previously diagnosed as being of actinomycotic origin. The organism in all instances appeared to be identical with a leptothrix isolated in 1896 by Piana and Galli-Valerio [16] from a dog which died of a suppurative pleuritis. They named their organism *Leptothrix pleuriticus*, but considered it identical with an-

other organism named *Discomyces pleuriticus* by Rivolta twelve years previously. In 1927,[17] and again in 1930,[18] Galli-Valerio and Bornand reported more cases of infection by *Leptothrix pleuriticus*. In two of their total of nine cases attempts to grow the organism succeeded.

There have been reported a number of cases of meningitis with the recovery from the spinal fluid, and in some instances from the blood also, of a Gram-negative, pleomorphic, coccobacillary organism which formed filaments varying in length. These have been considered as leptothrices by the various authors, but in discussing these cases Mackenzie [19] says: "All these communications however afford no conclusive evidence that the filamentous organism belongs to the higher group of bacteria." However, in cultures of a series of twenty-five cases of meningitis caused by Gram-negative organisms other than the meningococcus, Mackenzie six times isolated an organism which he considers a true leptothrix. The organisms all belonged to one species and appeared in two phases, (*a*) a presporing or non-sporing phase, and (*b*) a sporing phase.

From this review of the literature one can see that leptothrices have been discovered in the liver in only two instances. In neither of these was it proved that a leptothrix was responsible for the associated hepatic lesions. The case to be reported therefore appears to be the first recorded instance of leptothrical hepatic abscesses.

Report of Case

Clinical History: The patient was a girl of 11 years, native of a town in north Alabama, who while on a visit to a small town in south Tennessee began having chills about Sept. 1, 1930. During the next two weeks she had fever, and complained of weakness and pain near the right costal margin. She was at first thought to have malaria, but administration of quinine afforded no relief, and blood smears failed to show plasmodia. Typhoid was next suspected, and a Widal test is said to have been positive. The symptoms were not typical of typhoid, however, and during the latter part of September her temperature was normal in the morning with an afternoon rise to 104° F. The fever persisted, and she began having abdominal distention. This was at first intermittent, but ultimately became persistent. At the end of October abdominal paracentesis was performed and a large amount of fluid was withdrawn. The puncture wound drained for two weeks before healing. X-rays of the chest are said to have shown no abnormalities. During November her fever subsided somewhat, and she began to gain strength. She had no abdominal pain, bloody stools, diarrhea, or constipation. About December 1 she developed a cough and began expectorating large amounts of viscid yellow sputum. At the same time fever,

malaise and weakness increased, and she began losing weight. She was admitted to the Vanderbilt University Hospital Dec. 19, 1931. The past and family histories are irrelevant.

Physical Examination: The significant findings were as follows: Temperature, 102.4° F, pulse 132, respirations 36. She was emaciated (20 per cent underweight), and although she was in no apparent distress paroxysms of coughing were brought on by movement. The skin was rough and scaly, and brown in color. There was no glandular enlargement. The tongue was raw and beefy and covered by white patches, and the tonsils were enlarged and injected. There was flatness at the right lung base laterally and posteriorly, and many coarse and fine râles were heard in this area. The right cardiac border could not be made out, but the apex was pushed to the left. The heart rate was rapid, but the sounds were normal. The abdomen was distended and contained fluid. The liver extended 5 cm. below the costal margin and the spleen was easily palpated.

Laboratory Findings: Urine: Specific gravity 1.020 to 1.030, reaction acid, albumin 0, sugar 0, occasional white blood cell, no red blood cells or casts.

Blood: Red blood cells 3,050,000, white blood cells 13,600, hemoglobin 38 per cent. Differential count (supravital) showed neutrophils 75 per cent, lymphocytes 19 per cent, monocytes 6 per cent. Wassermann and Kahn negative.

Of four blood cultures two were sterile and two contaminated. Agglutination of serum for typhoid was slight twice (1:40 dilution) and negative once; negative for *Paratyphoid A* and *B*, melitensis, abortus, tularense and Weil-Felix.

Stools showed no parasites or ova. Tuberculin negative with 2.5 mg.

X-ray of the chest showed an abscess at the right lung base with infiltration of the surrounding parenchyma. The cardiac shadow was enlarged to both right and left.

Subsequent Course: On the day after admission 700 cc. of clear yellow fluid with a specific gravity of 1.013 were removed from the abdomen. The cell count was 1800 erythrocytes and 5400 leukocytes; of the latter 95 per cent were lymphocytes, 4 per cent polymorphonuclear neutrophils, and 1 per cent monocytes. No organisms were seen in smears or cultures. With institution of postural drainage of the lung abscess a small amount of non-malodorous pus was obtained and the cough became less severe. Eleven days after admission dilated veins were noted over the chest. At this time an X-ray following pneumoperitoneum showed adhesions between the right diaphragm and dome of the liver. The abscess cavity was poorly visualized, and there was a small amount of fluid in the right pleural cavity. The general condition improved somewhat, although there was no gain in weight. Fluid reaccumulated in the abdomen, and at the end of the third week 1500 cc. were removed. Subsequently no more fluid collected. The temperature was always elevated and showed much irregularity; it was usually above 100° F, and varied 2 degrees or more daily. The 8.00 A.M. or noon temperature was usually the lowest, and the 8.00 P.M. or midnight temperature the highest. During the fifth and sixth weeks it showed little variation, and was frequently below 100, but it again became irregular and rose above 103° F several times thereafter. X-ray of the chest at the end of the fourth week showed only the medial wall of the abscess with no longer a definite cavity. Another chest plate taken at the end of the sixth week showed little change in the lung, but much diminution of the cardiac shadow. By this time the child felt much better, and although she still had a high fever she was discharged after seven weeks in the hospital. Six days later she was brought back to the hospital in a moribund state and died a few minutes after being put to bed.

Autopsy Findings

Gross Description: The child was much undernourished and the layer of subcutaneous fat was very thin. The skin was dry and had a grayish sallowness which did not appear to be icteric. There were a few dilated veins over the chest anteriorly. The chest was pyramidal in shape, the lower portion flaring out markedly. A couple of needle holes were seen over the heart, the result of a postmortem pericardial tap which produced nearly 300 cc. of purulent fluid. The liver edge extended 8 cm. below the costal margin in the midclavicular line.

Peritoneal Cavity: There were approximately 200 cc. of clear, straw-colored fluid in the peritoneal cavity. The liver was greatly enlarged and its margins were smooth and rounded. In addition to extending downward lower than normal it caused some elevation of the diaphragm. The omentum was reflected up over the anterior-superior surface of the liver, being adherent to it at places; it enveloped the stomach and spleen, and was closely adherent to the latter. The liver and spleen were adherent to each other, and between them was a small amount of yellowish mucoid pus. There were many fibrinous and fibrous adhesions about this abscess and over the surfaces of the spleen and liver, holding these viscera to the diaphragm. A few fibrinous adhesions were present about the pelvic organs, but the organs themselves did not appear abnormal.

Pleural Cavities: There was no fluid in either pleural cavity. Fibrous adhesions held the right lung to the diaphragm, and fibrinous adhesions were present between both lungs and the pericardium, and the diaphragm and left lung base.

Pericardial Cavity: The pericardium was thickened, and when the pericardial sac was opened a shaggy deposit of fibrin was seen on all the pericardial surfaces with thick festoons connecting the visceral and parietal layers (Fig. 1). Approximately 100 cc. of thick, yellow, purulent fluid remained in the pericardial sac. In the anterior portion of the pericardium near the apex of the heart was a small circumscribed collection of slightly yellowish mucoid pus from which smears were made. The thoracic viscera, diaphragm and liver were removed in one piece.

Heart: A section through the ventricles revealed a very pale pink, soft myocardium. Further examination was not made as it was thought desirable to save the specimen intact.

Lungs: In general the lung tissue was normal, although there was some atelectasis. A small area of consolidation was found at the base of the right lower lobe, obviously the residuum of the lung abscess, as comparison with the roentgenograms showed. A fortunately directed section passing through the liver, diaphragm, and right lung base showed both the nature and manner of origin of the former lung abscess (Fig. 2, inset). An abscess in the upper part of the liver ruptured through the diaphragm into the pleural cavity, producing an adhesive pleuritis. Radiating from the thickened pleura into the lung was an area of fibrosis. Within the lung, pleura and diaphragm were small yellow flecks similar to those to be described in the liver.

Gastro-Intestinal Tract: Not remarkable.

Liver: When the common bile duct was cut a light yellow mucopurulent material escaped. Pressure upon the gall-bladder caused the escape of thick, dark brown bile. The gall-bladder wall was edematous. Several superficial abscesses were found in the liver by palpation, and a yellowish mucopurulent material was aspirated from one and used for cultural study. Sections through the liver revealed the presence of a great many abscesses varying in diameter from a few millimeters to 3 cm. These abscesses were grouped irregularly in the upper portion of the liver, and were abundant in both right and left lobes. A peculiarity of their distribution is that although the abscesses were very numerous in the lateral half of the right lobe and extended down quite far toward the inferior margin, in the medial half of this lobe they were much less numerous and were found only in the upper portion. There was, however, a continuous chain of abscesses extending from the outermost edge of the right lobe to the outermost edge of the left lobe. All these abscesses appeared to be in association with bile ducts and contained a light yellow, or grayish mucoid pus. The pus was perfectly smooth and contained no granules. When the pus was permitted to escape from them the majority of the abscesses were seen to have irregular walls, while the larger spaces had smooth, glistening walls and were obviously greatly dilated bile ducts. The walls of both abscesses and bile ducts were stained dark green by bile. The abscesses and, to a less marked degree, the dilated, pus-filled bile ducts were bordered by an irregular zone of bright yellow or orange tissue (Figs. 1 and 2). A section passing through the left lobe of the liver, diaphragm and

pericardium near the apex of the heart showed a large abscess between the pericardium and liver capsule, the diaphragm in this area having become necrotic and disappeared (Fig. 1). The liver capsule was much thickened. Within the abscess were many shaggy bands of fibrin surrounded by a thick, white, mucoid pus. Numerous abscesses were present in the upper part of the left lobe, and a communication could be demonstrated between one of these liver abscesses and the diaphragmatic abscess. The pericardium about this abscess was thickened in some places and quite thin in others. Although no fistulous connection between the pericardial cavity and the diaphragmatic abscess was found, it was obvious that the infection extended from the abscess through the pericardial sac to the pericardial surfaces. Although the sinusoids were congested, the liver tissue surrounding the abscesses was pale and whitish pink in color.

Spleen: The spleen weighed 270 gm. It was deep reddish purple in color and contained numerous, small, blackish areas of hemorrhage. There was some increase in connective tissue. No abscesses were seen.

Kidneys: The kidneys weighed 160 gm. each. They were enlarged and soft. The cut surface bulged outward markedly and showed extreme congestion. The ureters and bladder were normal.

The pancreas, adrenals and genital organs were not remarkable.

Microscopic Examination

Heart: The surfaces of the visceral and parietal pericardium are greatly thickened by a newly formed fibrous tissue with many fibroblasts and delicate collagen fibrils and numerous strands of unorganized fibrin. This tissue is very vascular and is infiltrated by moderate numbers of polymorphonuclear leukocytes, plasma cells, and a few large mononuclear leukocytes. Areas of hemorrhage are numerous in the parietal pericardium. The serosa is completely missing. Upon the superficial layer of the pericardium there is a thick, irregular layer of fibrin and many polymorphonuclear leukocytes (Fig. 5). A section taken through the abscess near the apex of the heart shows a much more chronic process than is seen elsewhere in the pericardium. The connective tissue is very dense and is infiltrated by large numbers of plasma cells, polymorphonuclear

neutrophils, and large mononuclear leukocytes. The diaphragm is also infiltrated by these cells. The muscle cells are atrophic and many of them have disappeared. The mononuclear leukocytes are greatly enlarged and have a foamy cytoplasm; they are abundant, and in many areas are the predominant cells. There are many foci in which tissue liquefaction has occurred, and about the margins of these foci is a zone of large mononuclear leukocytic infiltration.

Lungs: The sections show some collapse of the alveoli, with moderate numbers of polymorphonuclear leukocytes within the alveolar capillaries. A section from the left lower lobe includes the parietal pericardium. Between the visceral and parietal pleura is a small amount of fibrinous exudate containing a few polymorphonuclear leukocytes. The serosal cells are well preserved, and there is no organization of the exudate. Beneath the parietal pleura is the much thickened parietal pericardium which has already been described. Duplicates of this section stained by Goodpasture's carbol anilin fuchsin stain [20] show moderate numbers of leptothrices within the pericardial exudate (Fig. 7). Sections taken from the area of consolidation in the right lower lobe show great thickening of the alveolar walls, which in part results from fibrous tissue proliferation radiating outward from some of the bronchioles. Numerous foci of lymphocytic infiltration are seen. Some alveoli are completely effaced, but many are still patent, although nearly completely collapsed. These alveoli are lined by large cuboidal epithelial cells, and some contain a few polymorphonuclear leukocytes or a large mononuclear phagocyte with foamy cytoplasm. Some bronchioles contain many polymorphonuclear leukocytes and some desquamated epithelial cells. The pleura is much thickened by fibrous tissue proliferation and is adherent to the diaphragm, and within the pleura, diaphragm and bit of included liver are many groups of large, fat-laden mononuclear phagocytes identical with the occasional cell seen in the alveoli. These obviously gave rise to the yellow flecks seen in the gross. Duplicates stained by the carbol anilin fuchsin stain show many leptothrices within the purulent exudate in the bronchioles (Fig. 6), and some are seen within polymorphonuclear leukocytes. An occasional leptothrix is seen within an alveolus, but none is seen within the lung tissue or scar tissue.

Liver: In the region of the abscesses the liver capsule shows irregular areas of slight thickening in which are some new blood channels,

but neither fibrin nor leukocytic infiltration. The sinusoids are congested and many contain small thrombi of hyalinized fibrin. The cells are atrophic, particularly those about the central veins and adjacent to the abscesses and areas of fibrosis, and many cells contain small fat vacuoles. In some areas hemorrhage has occurred into the cords of cells, and in others necrosis of the cells is seen. The bile ducts appear to be increased in number, and there is a great increase in fibrous tissue about them. Polymorphonuclear leukocytes are seen within some of the ducts and in the surrounding connective tissue. This increase in connective tissue is marked in some areas, many whole lobules being entirely replaced, although in some instances a few liver cells persist. This tissue is infiltrated by large numbers of plasma cells, lymphocytes, neutrophilic and eosinophilic polymorphonuclear leukocytes, and large mononuclear leukocytes, and represents an effort at localization of the abscesses (Fig. 3). Many abscesses of uneven size and irregular outline are seen. The material within their centers consists mainly of polymorphonuclear neutrophils, but at their margins where tissue liquefaction has not yet occurred the other leukocytes just mentioned are abundant. The tissue at the periphery of these abscesses is infiltrated by large numbers of fat-laden, large mononuclear leukocytes (Fig. 4), many of which contain masses of pigment varying in color from dull yellow to orange, and some containing hemosiderin or hematoidin. The abscesses are obviously of various ages, some are very young, and others are healing. Sections stained by Goodpasture's carbol anilin fuchsin stain show moderate numbers of long bacillary forms and some filamentous organisms in the abscesses.

Spleen: There is a diffuse increase in the fibrous tissue of the spleen.

The gastro-intestinal tract, pancreas, adrenals, kidneys and bladder show no significant changes.

Comment: The essential features of this case consist of the presence of multiple liver abscesses, a diaphragmatic abscess and a subacute pericarditis, all demonstrated to be produced by a leptothrix, and a lung abscess unquestionably produced by the same organism. Rupture of a liver abscess led to formation of a subdiaphragmatic abscess with subsequent necrosis and disappearance of all the diaphragm in a rather large area. Although no actual communication between the diaphragmatic abscess and the pericardial cavity was

found, there can be no question that the pericarditis arose as a result of direct extension of the infection from the diaphragmatic abscess. Communication between the former lung abscess and a liver abscess was demonstrated, and although it is possible that the lung abscess was the primary focus of infection, it seems much more likely that it occurred as a result of rupture of a liver abscess. The source of the organism and the portal of entry to the body are obscure. No lesion of the gastro-intestinal tract was found which might have permitted invasion by the organism; however, there may have been such a lesion which had healed and left little or no trace of its presence.

Bacteriology

Smears: Smears of pus from the pericardial and liver abscesses showed many leukocytes and a few delicate Gram-negative filaments which showed no branching and varied from 20 to 150 microns in length, the majority measuring around 100 microns. Many of these filaments were beaded and a few were relatively thick, tapering off at each end. A few examples of phagocytosis of these filaments by polymorphonuclear leukocytes were seen. No other microörganisms were present.

Cultures: Aerobic cultures were made of the pus obtained from the liver abscess, the following media being used: yeast broth, ascitic fluid, glucose broth-ascitic fluid, blood agar plate, Sabouraud's plate, Sabouraud's stab and agar stab. An anaerobic culture with yeast broth and $\frac{1}{3}$ cc. of pus was also made. Incubation was carried out at 36° C unless otherwise noted. The glucose broth-ascitic fluid tubes received a heavy inoculation, and a few organisms were later found in smears from one of the two tubes, a small amount of growth presumably having occurred. After 3 days incubation a small amount of growth was noticed in the anaerobic tube. After a couple of days the growth was more abundant, but heavy growth never occurred. Smears showed long filaments identical with those seen in the smears of pus. Anaerobic subcultures of this tube were made using yeast broth and yeast broth plus ascitic fluid with vaseline seal, and aerobic cultures using blood agar plates, Sabouraud's medium, ascitic fluid with glucose, litmus milk, potato, and potato in yeast broth. Growth occurred in none, and anaerobic subcultures were again tried, using yeast broth with vaseline seal to which a small

piece of fresh kidney had been added. Growth occurred in this medium fairly well, the organism appearing as a flocculent mass at the bottom of the tubes. The sediment from two of these tubes was inoculated subcutaneously into each of two guinea pigs (Nos. 3 and 4) with results to be discussed more fully later; suffice it to say at present that abscesses developed and that pus was taken from one of these (Guinea pig 3) and cultured anaerobically with recovery of the organism.

The original culture was carried through 5 generations and the strain was then discarded, since a contaminating organism had been introduced by the fresh tissues used. A repetition of this accident was avoided by heating the tubes to 60° C for 30 or 45 minutes immediately after addition of the tissue. This heating was also intended to stop enzymotic action. Liver was at first used as well as kidney, but its use was not continued as fresh liver is frequently not sterile, and the introduction of contaminated tissue was avoided as much as possible. Subsequently only kidney was used. This medium permitted fairly free growth of the organism, but was rather unsatisfactory because of the precipitate formed as a result of heating it to ensure sterility; this precipitate was rather abundant and concealed the amount of growth of the leptothrix. Subcultures of the original strain, which we shall call Strain 1 for convenience, were made using five blood agar plates, two Sabouraud's medium plates, two flasks of bread medium (toasted bread crumbled with enough saline added to make a firm gell after sterilization) and one glucose agar plate containing ground up liver, all kept in an atmosphere of carbon dioxide. No growth occurred in any of these. One Sabouraud's plate in CO_2 was kept at room temperature but showed no growth. Two Sabouraud's plates and two blood agar plates were incubated in a chamber which had been exhausted to a pressure of 25 mm. of Hg and in which the remaining oxygen was removed by the use of pyrogallol; no growth occurred here either. A third flask of bread medium was incubated aerobically, but no growth occurred. All these media were heavily inoculated, the inoculum being obtained from tubes of liver or kidney broth in which the strain was maintained. This strain was still growing vigorously at the time it was discarded, the contaminant which it had acquired apparently having no effect on its growth. Inasmuch as the organism had been meanwhile obtained in pure culture again and was growing vigor-

ously, no advantage was seen in maintaining the contaminated strain further. All but one of the subsequent cultures were anaerobic.

Pus from the abscess in Guinea pig 3 was inoculated into two tubes of broth, three tubes of liver broth, and two tubes of kidney broth. The plain broth tubes received the heaviest inoculation, and after 48 hours many small round colonies were seen on the side of one tube (Fig. 8); the other tube contained only two colonies, but these grew much larger than those in the first tube. Only one of the five tubes containing tissue showed growth, but when the others were reinoculated with colonies from the plain broth tubes abundant growth occurred. The strain so established we shall call Strain 2. This strain was carried through 16 generations and furnished material for most of the animal inoculations. In addition to the various culture media which have been previously mentioned as having been used, the following were tried with this strain of the organism: Dorsett's egg medium (aerobic and in CO_2), yeast broth with egg yolk, and glucose broth with egg yolk; the effect of addition of glucose to the broth and tissue was also tried. No growth was obtained on Dorsett's medium. The second egg medium gave good growth if pus and glucose were present, but it was unsatisfactory because of the sediment in it. Glucose seemed to retard rather than to augment growth except in the egg yolk medium, growth not occurring in its absence in this medium.

A third strain was recovered from a subcutaneous abscess produced in Guinea pig 7 by Strain 2. This strain was carried through 5 generations.

Characteristics of the Organism

A. Morphological: The organism seen in smears of the cultures was unquestionably the same as the one seen in smears of the pus. It was Gram-negative, and stained poorly with most stains. Dilute carbol fuchsin gave the best results, although Wright's stain and Loeffler's methylene blue gave good results when prolonged staining was employed. Many filaments were beaded, but none appeared to be formed of strings of bacilli. Wright's stain brought out the beading extremely well, but it was still quite conspicuous in carbol fuchsin and methylene blue stains. The filaments did not bend sharply, but formed smooth sinuous curves. No clubbing of the ends of the filaments was noted, but some very thick fusiform filaments

were seen (Fig. 14). In one smear stained by Wright's stain, what were at first thought to be spores were seen (Fig. 10); spore stains on other smears from this same culture showed that these structures were not spores — they appeared to arise as a result of longitudinal splitting of the filaments. Similar structures were not seen subsequently, and never again was anything suggestive of a spore seen. The filaments varied in length; some cultures showed many bacillary forms (Fig. 12) and others showed very long filaments (Figs. 9 and 13); many filaments were seen exceeding 300 microns in length. The bacillary forms were most numerous in media which offered less favorable conditions for growth. In no instance was unquestioned branching observed, although some instances were seen in which a short filament lay at right angles to another filament with its end touching the side of the other (Fig. 10). There is no doubt that the majority of these were only fortuitous. Where flocculent growth of organisms occurred they were pretty much matted together, and the colonies consisted of matted masses forming tough clumps which were mashed or teased only with some difficulty (Fig. 9).

B. Cultural: The best growth was obtained in yeast broth containing a small piece of fresh kidney; whether heating made this medium better or not is not known. A fairly good medium was found to be yeast broth to which sterile pus had been added, or pus containing the leptothrices. Some growth occurred under partial aerobic conditions, as evidenced in the glucose-broth ascitic fluid inoculated with pus, and also by a tube of broth containing kidney which was inoculated and not resealed by vaseline. In these tubes bacillary forms of the organism predominated, but they were also numerous in the egg yolk-broth medium, and occasionally were very abundant in the broth-kidney medium. In the tubes of broth containing pus, discoid colonies were formed on the sides of the tubes. The colonies showed some variation in size, but averaged about $\frac{2}{3}$ mm. in diameter. Their centers were more transparent than their edges (Fig. 11), and radiating outward from their edges was a feathery tangle of filaments (Fig. 9). Generally growth occurred with colony formation on the tissue and on the sides of the tubes. The latter colonies were always small and were easily dislodged, many falling to the bottom of the tube at the slightest jar. The colonies growing on the tissue were not discoid in shape but grew out equally in all directions, forming hemispheres. They were somewhat

less tough than the colonies formed in the pus broth tubes, and they reached a considerably larger size, one of them reaching 5 mm. in diameter. The colonies in the tubes grew to larger sizes when only a few colonies were present. When subcultures were made, one of the colonies was fished out with a loop and rubbed on the side of the tube to be inoculated in order to break up the colony as much as possible. Since some colonies were more easily broken up than others, there was much variation as to the amount of breaking up that occurred. In some tubes colony growth did not occur so readily, a more flocculent type of growth being found. It seems possible that this may have resulted from more thorough breaking up of the inoculum, although it is probable that many colonies were formed in these tubes and concealed by the precipitate within them. Subcultures of a culture kept at room temperature for 1 month failed to grow. Subcultures of a culture refrigerated 6 months showed no growth.

Summary of Cultural Characteristics: Fairly free growth occurred only under "anaerobic" conditions in yeast broth containing pus, or fresh or heated animal tissue. Growth was not obtained on solid media. Occasionally flocculent growth occurred, but usually definite colonies were formed. Under less favorable conditions bacillary forms were abundant.

Animal Inoculation

One-half cc. of pus from the liver abscess was given to each of two rabbits, two guinea pigs, and two baby rats. One rat received intraperitoneal, and the other intrapleural injection, but showed nothing. The guinea pigs were inoculated subcutaneously; one pig showed only some induration about the site of inoculation for several days, but the other developed a small firm papilla which began to recede after 9 days, and 4 days later had disappeared; 3 days still later all induration had disappeared. One rabbit received the pus intravenously, and the other received it intraperitoneally, an attempt being made to puncture the liver and inject the pus into it. The first rabbit showed no fever at any time. Three weeks after inoculation it was killed, but autopsy revealed nothing of interest. The second rabbit was killed after a month but nothing of interest was seen.

Meanwhile the organism had been found in the anaerobic culture of the pus and had been transferred to several tubes of broth-kidney medium. After several days growth the tissue was removed from two tubes and the tubes centrifuged; most of the supernatant fluid was poured off and the sediment used for inoculation. One guinea pig (No. 3) was inoculated subcutaneously with the material from a 7 day culture, and developed a large firm nodule at the site of injection. Sixteen days later it was killed and nothing of note was found except an encapsulated abscess at the site of inoculation. This contained a yellowish white pus; smears contained leptothrices, and anaerobic cultures contained many colonies (establishing Strain 2). The second tube, an 11 day old culture, was injected subcutaneously into another guinea pig (No. 4), two sites of inoculation being chosen, and two nodules appearing. After 3 days the nodules diminished in size, and after 2 weeks had disappeared. At the same time two baby rats and one mouse were given some of the culture subcutaneously, but showed no reaction. Subsequently three more guinea pigs received cultures subcutaneously. Nodules formed as before, and after 16 days pus was aspirated and cultured from one pig, establishing Strain 3. The nodules in all three gradually diminished and disappeared after about a month. A rabbit was inoculated subcutaneously with material from two cultures. Fourteen hours after inoculation a large vesicle appeared but rapidly receded; underneath it however was an area of induration which gradually developed into a nodule nearly 1 cm. in diameter. Seventeen days later aspiration was attempted, but failed because of thickness of the contents. Thirty-eight days after inoculation the abscess ruptured spontaneously.

Intraperitoneal inoculations with cultures were tried with three baby rats and two guinea pigs. The rats died 2 days later and showed nothing. The guinea pigs appeared very ill for a few days, but rapidly recovered and were apparently normal a week later. One of the two pigs was killed 12 days after inoculation. A small globule of pus was found free in the peritoneal cavity, and another was found attached to the wall of the seminal vesicle. A culture of the pus produced many colonies. This strain was subcultured but failed to grow. It was thought that if a sterile peritonitis could be produced it might be possible to establish the leptothrix within the peritoneum. Accordingly a guinea pig was given some aleuronat in-

traperitoneally shortly before the culture was given. The animal became very ill, but soon recovered. A mass was palpable in the abdomen, and after 25 days the pig was killed. Several loops of small intestine and the seminal vesicles were adherent to each other and to the anterior abdominal wall. A small yellow spot was found on one seminal vesicle. This was cultured, but no growth occurred.

Intravenous injection of cultures was tried with two rabbits. The first received all the material in two 4 day old cultures and had a diarrhea for a week, but never developed fever. Eighty-one days later the animal was killed, but the tissues showed nothing of note. The second rabbit received a single colony which measured 5 mm. in diameter, the remainder of the material in the tube being rejected. This animal showed no reaction of any kind.

Intrahepatic injection of material from three cultures was carried out on one rabbit. The abdominal cavity was opened and the liver injected in two places. Some leakage was seen to occur at the sites of injection. One month later the animal was killed, but no definite lesions were found.

An attempt to enhance the virulence of the organism by rapid passage from animal to animal met with early defeat. Subcutaneous inoculation of one guinea pig with pus removed from an abscess produced by injection of a culture of the organism in another pig resulted in the formation of a small nodule which very soon disappeared.

As controls each of two guinea pigs was injected subcutaneously with the sediment in two tubes of unused sterile broth-kidney medium. One showed no reaction, and the other developed a small nodule which very soon disappeared.

Summary of Results of Animal Inoculation: Abscesses were produced in guinea pigs and rabbits by subcutaneous inoculation of cultures. Intravenous and intraperitoneal injection of cultures caused only transitory indisposition of the animals.

Discussion

The amount of experimental work done with this organism is admittedly inadequate. More extensive cultural studies including fermentation reactions with sugars were planned, but could not be done because of the special growth requirements of the organism.

An attempt to increase virulence by rapid passage from animal to animal was abandoned when it was found that the number of organisms in the abscesses formed were not adequate to produce anything other than a very slight reaction in a second animal. Direct inoculation into the liver gave equivocal results and was not repeated. Peritonitis could not be produced, although subcutaneous inoculations were successful. The results obtained are not without value however, and may offer help whenever a similar organism is recovered in the future. The organism recovered from this case is obviously different from all pathogenic leptothrices which have been heretofore described, and there is no evidence to indicate that any two of the leptothrices previously reported to be pathogenic to man are the same. The leptothrix producing the disease in this case seems to have a higher pathogenicity for man than those previously encountered, as evidenced by the intense reaction to the presence of relatively few organisms. The fatal outcome of the case is another evidence of high pathogenicity, although the presence of the severe pericarditis makes it impossible to say whether or not the child would have died because of the liver abscesses. It is quite possible that she would not, for many liver abscesses were healing and the lung abscess healed nearly completely.

Summary

1. A summary of the literature pertaining to leptothrices pathogenic to man is presented.

2. Report is made of a case of leptothricosis in which multiple liver abscesses were formed, with rupture of one abscess into the base of the right lung and formation of a lung abscess, and rupture of another in the left lobe of the liver, leading to the production of pericarditis.

3. The organism was isolated in pure culture from the liver and successfully carried *in vitro* through many generations. No other organisms were present in the lesions.

4. Experimental work with the organism showed it to be slightly pathogenic for rabbits and guinea pigs.

5. This organism has apparently never been previously encountered.

REFERENCES

1. Gruber, G. B. Knötchenbildungen um Parasiteneier. Handbuch der speciellen pathologischen Anatomie und Histologie, by Henke, F., and Lubarsch, O. J. Springer, Berlin, 1930, **5**, Pt. 1, 525–534.
2. Huntemüller, O. Handbuch der pathogen Mikroörganismen, by Kolle and Wassermann, Ed. 3, **5**, Pt. 1, 1–16.
3. von Beust, Th. Die pleomorphen fadenbildenden Organismen des Zahnbelages und die fusiformen Bacillen. *Berl. klin. Wchnschr.*, 1909, **46**, 2058. (Cited by Huntemüller.)
4. Mendel, J. Leptothrix astéroïde. *Compt. rend. Soc. de biol.*, 1918, **81**, 471–475.
5. Flexner, S. A study of the Bacillus (Leptothrix?) pyogenes filiformis (Nov. Spec.) and of its pathogenic action. *J. Exper. Med.*, 1896, **1**, 211–280.
6. Follet and Sacquépée. Zona métamérique. Infection par un Leptothrix. *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1902, **19**, S. 3, 573–575.
7. Blumer, G., and MacFarlane, A. An epidemic of noma; report of 16 cases. *Am. J. M. Sc.*, 1901, **122**, 527–536.
8. Mathews, P. A case of localized empyema associated with the presence of a leptothrix. *Practitioner*, 1905, **74**, 197–202.
9. Schottmüller, H. Zur Ätiologie und Klinik der Bisskrankheit. (Ratten-, Katzen-, Eichhörnchen-Bisskrankheit.) *Dermat. Wchnschr.*, 1914, **58** (Suppl.), 77–103.
10. Deussing, R. Leptothrixangina mit akuter Glomerulonephritis. *Deutsche med. Wchnschr.*, 1920, **46**, 1278–1279.
11. Roedelius, E., and Brack, E. Leptothrix-Mykose der Zunge. *Arch. f. klin. Chir.*, 1926, **141**, 1–13.
12. von Meyenburg, H. Über einen Fall von Fadenpilzgeschwüren der Magenschleimhaut mit metastatischen Abszessen in der Leber. *Virchows Arch. f. path. Anat.*, 1921, **229**, 30–39.
13. Lang, F. J. Durch Leptothrix — Infektion bedingte Leberinfarcierung in einem Falle von callösem Ulcus Duodeni. *Virchows Arch. f. path. Anat.*, 1921, **234**, 367–377.
14. Galli-Valerio, B. Parasitologische. Untersuchungen und parasitologische Technik. *Centralbl. f. Bakteriol.*, 1915, Orig. **76**, 511–518.
15. Galli-Valerio, B. Parasitologische Untersuchungen und Beiträge zur parasitologischen Technik. *Centralbl. f. Bakteriol.*, 1917, Orig. **79**, 41–48.
16. Piana, and Galli-Valerio, B. Cited by Galli-Valerio and Bornand.
17. Galli-Valerio, B., and Bornand, M. Le Leptothrix pleuriticus (Rivolta) Piana et Galli-Valerio chez l'homme. Arb. ü. Tropenkrankh. (Festschrift B. Nocht), Hamburg, 1927, 134-138.
18. Galli-Valerio, B., and Bornand, M. Etudes sur les leptothricètes et les actinomycètes. *Schweiz. med. Wchnschr.*, 1930, **60**, 179–182.

19. Mackenzie, I. Pathogenic leptothriceae. In "A System of Bacteriology in Relation to Medicine." Medical Research Council, Great Britain. London, 1931, **8**, 91–100.

20. Goodpasture, E. W. A study of rabies, with reference to a neural transmission of the virus in rabbits, and the structure and significance of Negri bodies. *Am. J. Path.*, 1925, **1**, 547–582.

DESCRIPTION OF PLATES

PLATE 12

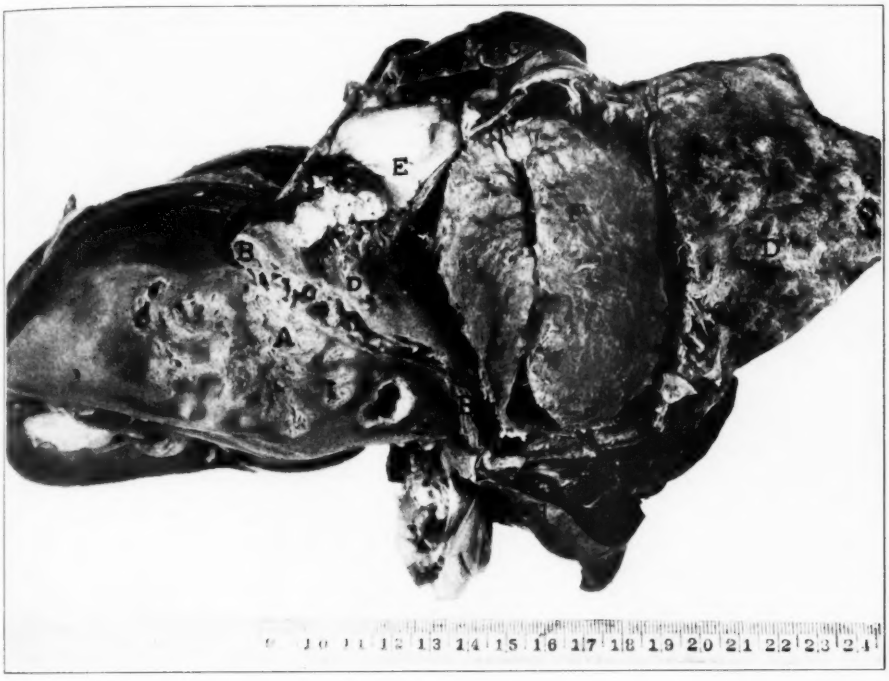
FIG. 1. Thoracic viscera and liver. The normal relations have been somewhat distorted in fixation.

A = cut surface of left lobe of liver showing multiple abscesses. B = diaphragm. C = abscess which has destroyed a portion of the diaphragm. D = inner surface of the parietal pericardium. E = outer surface of pericardium. F = heart. G = right lung. H = left lung.

FIG. 2. Right lobe of liver and right lung base.

The large photograph shows the numerous abscesses, dilated bile ducts, and the yellow and orange flecks in the liver. Infiltration of the lung base is also seen.

The inset shows the reverse surface of this slab, about 1 cm. lateral to the other surface. Note rupture of abscess through the diaphragm, adhesive pleuritis and extension of infection into lung.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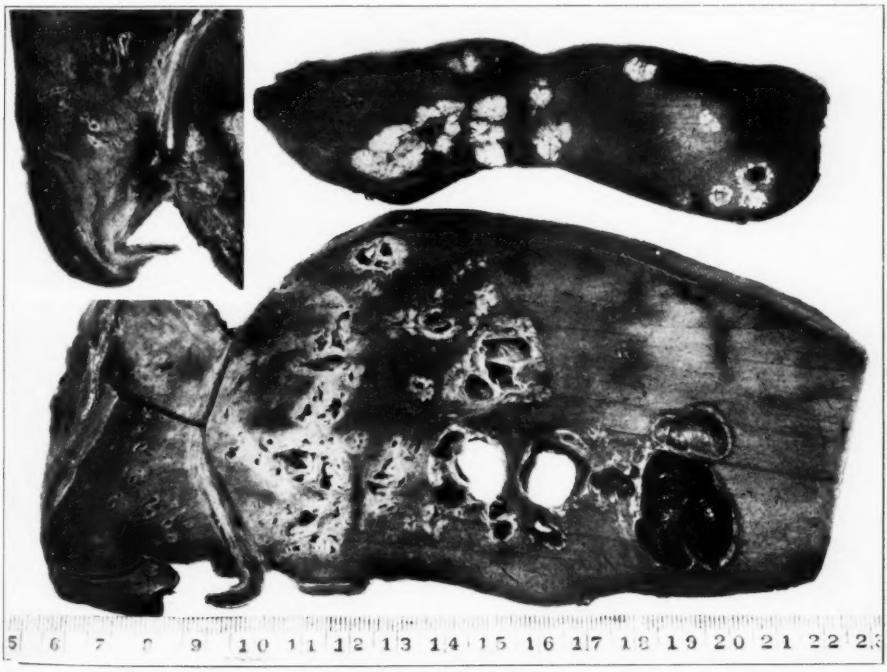
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PLATE 13

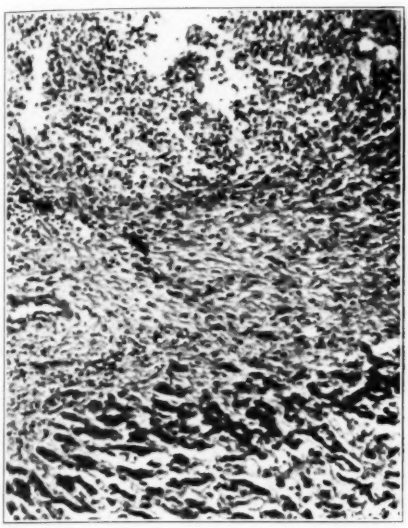
FIG. 3. Liver. Abscess surrounded by large amount of fibrous tissue. Hematoxylin and eosin stain. × 80.

FIG. 4. Liver. Edge of large abscess with numerous fat-laden monocytes at outermost border. Hematoxylin and eosin. × 80.

FIG. 5. Pericardium. Serofibrinopurulent exudate into pericardial cavity above, granulation tissue of thickened pericardium below. Hematoxylin and eosin. × 80.

FIG. 6. Leptothrices in exudate in bronchus at right lung base. Carbol anilin fuchsin stain. × 2000.

FIG. 7. Leptothrix in pericardial exudate. Carbol anilin fuchsin stain. × 2300.



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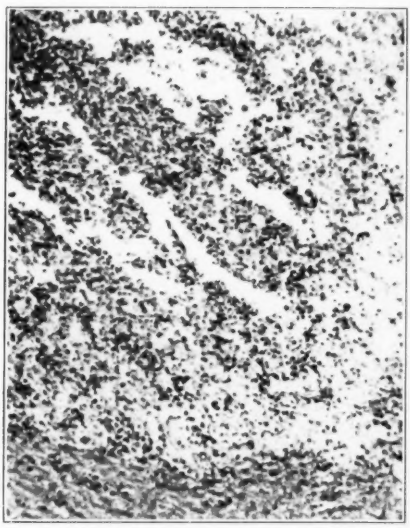

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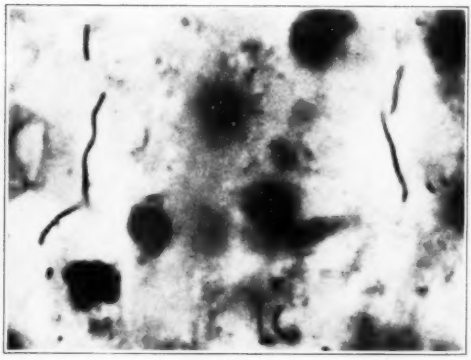

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Harris

Multiple Liver Abscesses Caused by a Leptothrix

PLATE 14

FIG. 8. Test tube containing numerous colonies of leptothrices. Culture of pus from subcutaneous abscess of guinea pig. × 1.8.

FIG. 9. Edge of one of the colonies in Fig. 8 illustrating the tangle of filaments with outward radiation of single filaments. Unstained. × 800.

FIG. 10. Smear of culture showing structure at first thought to be a spore. This organism also shows one of the very rare examples of what may be branching. Wright's stain. × 2300.

FIG. 11. Colony from Fig. 8 showing pale center and radiation of filaments out from the edge. Unstained. × 120.

FIG. 12. Smear of culture showing bacillary forms. Dilute carbol fuchsin. × 2300.

FIG. 13. Smear of culture showing beading of filaments, and variation in thickness and density of staining of various filaments. Dilute carbol fuchsin. × 2300.

FIG. 14. Smear of culture showing type of thick fusiform structure occasionally seen. Observe the tangled mass of long filaments and beading of some filaments. Dilute carbol fuchsin. × 2300.

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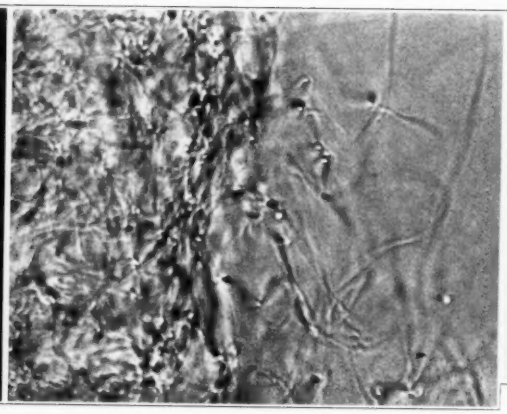

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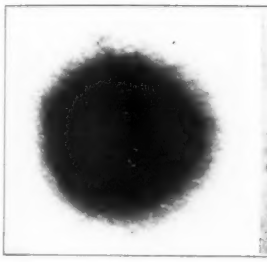

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Multiple Liver Abscesses Caused by a Leptothrix

HISTOLOGICAL STUDY OF A CASE OF THE EASTERN TYPE OF ROCKY MOUNTAIN SPOTTED FEVER *

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Although the disease now known as Rocky Mountain spotted fever had undoubtedly existed in Montana and Idaho for years, it began to receive attention only forty years ago, and it was not until 1896 that it was first described as a clinical entity.[1] Cases of the disease were soon recognized in other western states. The first case seen east of the Rockies was reported by LaBier in 1925.[2] The patient was a child, native of Terre Haute, Indiana, who was bitten by a "bug" which, although not identified, was presumably a tick. The child developed a disease clinically similar to Rocky Mountain spotted fever and eventually recovered. The Weil-Felix reaction was not determined. It remained, however, for Dyer, Rumreich and Badger [3, 4, 5] in 1931 to establish upon an immunological and clinical basis beyond all doubt the existence of Rocky Mountain spotted fever in the eastern United States. In 1926 Maxcy [6] demonstrated the existence of endemic typhus in the southeastern states, and since the publication of his studies cases of spotted fever have doubtless been erroneously regarded as typhus.

In an analysis of 100 selected cases separated on the basis of epidemiological considerations into two groups of equal size, the essential differences between the two diseases as enumerated by Dyer and his coworkers [4, 5] are as follows: Cases of endemic typhus were predominantly of urban origin and occurred in association with rat infestation. Rocky Mountain spotted fever occurred in persons living in rural sections, or having rural contact, on premises as a rule rodent-free, and in a significantly high percentage of cases followed shortly on tick bite. As well as could be estimated, the incubation period of typhus was seven to fourteen days, while that of spotted fever was usually less than a week. The rash in typhus appeared first on the chest and abdomen, over the legs anteriorly and laterally, and frequently on the medial surface of the upper arms. In many

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cases there was no further extension, in a rather large number the back was next involved, and in the more severe cases the eruption became generalized. The face was always free, and in only one case of their series were the palms and soles involved. The rash was macular, rose or dull red in color and faded on pressure. In some cases many lesions were papular. The rash persisted for two to nine days and then rapidly disappeared. In spotted fever the rash nearly always was first seen on the wrists and ankles; it appeared next on the back, and rapidly became generalized. The palms and soles were frequently, and the face occasionally, involved. The lesions were at first roseolous macules, but by the middle of the second week they had become petechial in all but the mildest cases. At this stage and subsequently the rash was purpuric and as a rule most abundant on the wrists and ankles, legs, upper part of the back, shoulders, lateral surfaces of the arms, chest, abdomen, palms and soles, and face — in the order mentioned. Petechiae, when numerous, often became confluent. Evidence of the rash sometimes persisted for several weeks as dusky purplish or yellowish brown spots. In 36 per cent of the spotted fever cases the spleen was enlarged, and was usually tender; it was palpable in two and tender in one of the typhus group. Rigidity of the neck with presence of Kernig's sign was noted in many of the spotted fever cases, but in only one case of typhus. A small ulcer at the site of the tick bite, with enlargement of the regional lymph glands, was occasionally noted in spotted fever. Disturbances of the central nervous system were much more severe in spotted fever. In both diseases lethargy, often associated or alternating with insomnia, restlessness or irritability, was present in nearly all cases. Stupor was more frequent and coma was present, only in spotted fever. Meningismus was frequent in spotted fever and sometimes there was violent delirium. In endemic typhus the leukocyte count was generally normal or moderately diminished, while definite leukocytosis was present in spotted fever. In the endemic typhus series there were no secondary cases in any family. In the Rocky Mountain spotted fever type group there were three instances of occurrence of multiple cases in a household. In one instance three cases occurred at intervals of twelve days and seven weeks. In each of the other two there were two cases which occurred within a week of each other. Cases of typhus were rare among children, but spotted fever cases among children were more numerous. No deaths oc-

curred in the typhus group, while seven deaths occurred in the spotted fever group. Of 93 cases of spotted fever occurring in five states and the District of Columbia during the spring and summer of 1930 death occurred in twenty-one instances (23 per cent).

Until the appearance of Wolbach's studies on Rocky Mountain spotted fever [7] knowledge of the pathology of the disease was very inadequate. Wolbach collected reports of fifteen autopsies on spotted fever cases and added five more. In approximately ten of these twenty cases the central nervous system was examined and found to be free of lesions. Because of this the presence of brain lesions has been considered as of great diagnostic value in differentiating typhus from spotted fever. This criterion has recently been shown to be inaccurate in so far as the Eastern form of spotted fever is concerned. Dyer, Rumreich and Badger [3, 5] found brain lesions in approximately half of their guinea pigs and in two monkeys which had been inoculated with blood from cases of the Eastern form of Rocky Mountain spotted fever. Shortly thereafter Lillie [8] was able to demonstrate lesions in the brains of five people who died of a disease identified by Rumreich as being clinically and immunologically Rocky Mountain spotted fever. Lillie gives a full account of the pathology of these cases and compares it with the pathology of the Western form of spotted fever and with that of European typhus. The lesions observed in our case are nearly identical with those described by Lillie.

Report of Case

Clinical History: The patient was a white girl of 5 years, native of a small town in west central Tennessee, who was admitted to the Vanderbilt University Hospital June 4, 1931 for diagnosis.

Seven days before admission she developed fever, was sluggish and sleepy, and failed to get up out of bed in the morning. She remained in bed thereafter, had a temperature of 102 to 103° F, was drowsy or even stuporous during the day, and at night was restless and irritable. She occasionally complained of "stomach-ache," but only once complained of headache. Two days after the onset of illness an erythematous rash was noticed over the backs of the hands and lower legs. The rash rapidly became more extensive and soon spread to the body. During the last three days before admission she cried when moved, and for twenty-four hours her neck seemed stiff. Subsequently it was discovered that a tick had been removed from her shoulder about a week before she became ill.

Physical Examination: Temperature 105, pulse 152, respirations 36. The child was drowsy but rational, and much dehydrated. She was at times restless, and any handling seemed to hurt her. The skin over the arms, legs and trunk

was covered by a rash which in places was macular and faded somewhat on pressure, and in other places was petechial. The neck was slightly stiff, and Kernig's and Brudzinski's signs were present. The heart, lungs and abdomen were not remarkable.

Laboratory Findings: Urine: amber, acid, specific gravity 1.015, albumin 0, sugar 0. There was an occasional white blood cell; no red blood cells or casts were present.

The blood showed white blood cells 15,600. Hemoglobin 12.6 gm. Differential count: polymorphonuclear leukocytes 79 per cent, lymphocytes 20 per cent, monocytes 1 per cent. Wassermann and Kahn negative. Blood culture negative.

Subsequent Course: Lumbar puncture produced a normal fluid under slightly increased pressure. On the following day the rash had spread, and there were bright petechiae covering the chest, neck, face and arms. The face, hands and feet appeared swollen. She was stuporous and at times irrational. A second lumbar puncture revealed increased sugar but nothing else. The temperature meanwhile had varied between 100 and 104° F, but was usually around 102 or 103° F. Application of a tourniquet to the arm on the third day in the hospital caused the appearance of more petechiae. A stool contained changed blood. By this time the petechiae had darkened, some were confluent, and numerous bluish blotches appeared on the body. The edema became more marked and the eyes were swollen shut. Albumin appeared in the urine on the fourth day in the hospital. Weil-Felix reaction on blood drawn on the second day in the hospital was negative, but blood drawn three days later gave a positive reaction in a dilution of 1:40. Her condition failed to improve, and on the sixth day in the hospital the temperature rose to 105° F, and death occurred. A few râles were present in the lungs before death.

A 6 year old brother, who was bitten by a tick at the same time, became ill two days after his sister was admitted to the hospital. He appeared to have the same disease, but recovered.

Autopsy Findings

The skin showed innumerable petechiae, many of which had become confluent, forming large irregular splotches. The petechiae were most numerous in the locations in the order named, on the extremities, back, chest, abdomen, neck and face. There was also a generalized edema which was most marked in the neck and face, and particularly on the left side. Both eyes were chemotic and the lids swollen, the left being the more edematous. There was also more edema of the left arm and hand than of the right.

The peritoneal cavity contained nearly 300 cc. of clear yellow fluid. There were many small petechiae beneath the serosa of the intestines, and some large petechiae in the mucosa could be seen from the exterior. The abdominal lymph glands were enlarged and pink. The right pleural cavity contained approximately 40 cc. of clear

yellow fluid and the left pleural cavity 25 cc. There was a definite increase in pericardial fluid, about 35 cc. being present. The lungs showed rather numerous areas of bronchopneumonia. A caseous tubercle 3 mm. in diameter was found in the right lower lobe near the hilum. There were moderate numbers of petechiae in the mucosa of the stomach and small intestine; only a few were seen in the colon. No ulceration of the mucosa was seen. The liver showed little, other than a few small yellow tubercles. The heart, pancreas and adrenals appeared normal. The spleen was enlarged, weighing 100 gm., and the tissue was soft and deep purple in color. The kidneys were intensely congested, and several petechiae were seen in the pyramids, but none were seen in the cortex or pelves. The urinary bladder contained a few. The brain showed some flattening of the convolutions and intense congestion of the cortical vessels. The meninges and cerebrospinal fluid were normal. No abnormalities were seen on gross section of the brain.

Microscopic Examination

Heart: Some of the capillaries and many of the smaller arterioles are thrombosed. A few are occluded only by a hyalinized mass of fibrin, but included within the majority of the thrombi are polymorphonuclear and large mononuclear leukocytes. Many thrombi contain pyknotic nuclear fragments also. The surrounding tissue is infiltrated by large numbers of polymorphonuclear and large mononuclear leukocytes and plasma cells, and lesser numbers of lymphocytes (Fig. 9). The endothelial cells of many of the blood vessels are much enlarged, but only a few of the smaller vessels are occluded as a result of swelling of the endothelium. There are a great many leukocytes within the blood vessels of all tissues studied, and they are more numerous in the smaller vessels than in the larger ones. Many of the larger arteries show deposition of small masses of compact fibrin upon their inner walls, but no such vessel is seriously occluded by the thrombus — in fact these vessels show no reaction other than slight leukocytic infiltration about them. There is some myocardial edema. There are many foci in which a few myocardial cells are necrotic and invaded by polymorphonuclear and large mononuclear leukocytes. There are a great many foci not definitely associated with either acute myocardial necrosis or with thrombosis of the

blood vessels in which the interstitial tissue is infiltrated by large numbers of plasma cells and large mononuclear leukocytes and few lymphocytes. The endocardium and epicardium are normal.

Aorta: Normal.

Lungs: There are numerous areas of bronchopneumonia. Within three sections of lung are seen a few arterioles completely occluded by fibrin thrombi, and small, dense masses of fibrin are seen deposited upon the intima of a few other arteries.

Gastro-Intestinal Tract: Sections of the stomach, jejunum, ileum and colon show no mucosal lesions. However, within the submucosa and muscularis of each section there are several arteries which are partially or completely occluded by thrombi. The walls of these vessels are invaded by small numbers of leukocytes, some of which have pyknotic, fragmented nuclei. The endothelial cells of many of the unthrombosed vessels are swollen and the vessels contain many leukocytes. The tissue immediately about the thrombosed vessels and about some of the unthrombosed ones is infiltrated by plasma cells, large mononuclear leukocytes and a few mast cells; polymorphonuclear leukocytes are also seen in varying numbers about these vessels. An interesting finding observed to some extent in all tissues is the presence of pyknotic nuclear fragments within large mononuclear leukocytes in unthrombosed vessels. In the colonic submucosa are three longitudinally cut large arteries which have numerous thin patches of hyalinized fibrin deposited upon the intima; overlying the fibrin is a layer of thinned out endothelial cells, but there is no organization of the fibrin.

Abdominal Lymph Glands: Two small lymph glands are included in the section of stomach; the lymph sinuses are invaded by many large mononuclear leukocytes and polymorphonuclear leukocytes, many of which are phagocyted by the mononuclears. The germinal centers of some of the mesenteric lymph glands are hyperplastic. The sinusoids contain a few polymorphonuclear leukocytes, and many of these have been phagocyted by large mononuclears which are rather numerous. Within the reticulum of the glands are seen a few multinucleated cells only slightly larger than the average monocyte. Only a few thrombosed arterioles are seen within the glands, but within the surrounding connective tissue are many thrombosed arterioles with perivascular leukocytic infiltration.

Liver: There are a few tubercles in the sections, one of which is

calcified centrally. The liver cells have shrunken away from the sinusoidal walls and there is a small amount of granular precipitate in the resulting space. The sinusoids are much congested and contain many monocytes and polymorphonuclear leukocytes. The Kupffer cells are enlarged, and some show phagocytosis of erythrocytes and polymorphonuclear leukocytes. Some of the arterioles are partially or completely occluded by hyaline thrombi, and are surrounded by leukocytes. Small, hyaline, mural thrombi are seen in one portal vein and one hepatic vein. There is an infiltration of some of the portal areas by plasma cells and monocytes, even in the absence of arterial thrombosis. There are several foci of no constant distribution in which a few liver cells have become necrotic and invaded by polymorphonuclear leukocytes. A few of the liver cells contain fat vacuoles.

Pancreas: There are many foci in the pancreas in which the stroma is infiltrated by polymorphonuclear leukocytes, monocytes and plasma cells. Some of these are definitely associated with arterial thrombosis, but many are not. In many such areas there is also acute necrosis of the pancreatic alveolar cells, the insular tissue being uninjured. In addition to the numerous thrombosed small arteries, there are several large arteries which are partially, or completely occluded by fibrin. The fibrin in the partially occluded vessels is covered by a single layer of greatly attenuated endothelial cells and shows no organization.

Spleen: The splenic sinusoids are intensely congested and there are numerous areas of hemorrhage. There are many foci in which numerous polymorphonuclear and large mononuclear leukocytes are aggregated, and some of the sinusoids appear to be occluded by them. Some of the sinusoids are thrombosed. The monocytes show very active phagocytosis for red blood cells, polymorphonuclear leukocytes and lymphocytes. Although a few germinal centers show lymphoid hyperplasia the majority of the malpighian corpuscles are small, and there are few lymphocytes within the stroma.

Kidneys: There is moderate vascular congestion throughout the kidneys. Within the areolar connective tissue outside the pelvis and within the capsule there are numerous thrombosed arterioles with the perivascular infiltration previously mentioned. Within the pyramids are a great many foci of infiltration of the interstitial tissue by large mononuclear and polymorphonuclear leukocytes and

plasma cells. Most of these are definitely associated with thrombosed arterioles or capillaries. In the smaller and medium-sized foci there is no injury to the tubules (Fig. 11), and no leukocytes are seen within the tubules, but in the largest lesions some of the tubular epithelium is necrotic and the tubules are invaded by leukocytes. Some of the largest lesions have progressed nearly to the state of abscess formation (Fig. 10). Many of the larger lesions are associated with hemorrhage. Where the medullary rays are cut longitudinally it is seen that these foci of leukocytic infiltration radiate outward from the pelvis and are much longer in this diameter than they are transversely. The cortical tissue shows some parenchymatous degeneration, but is nearly free from lesions like those in the medulla. Except for a few focal lesions which are immediately adjacent to thrombosed vessels in the medulla or capsule, there are only two foci in the cortical tissue of two sections in which there is arteriolar thrombosis with perivascular leukocytic infiltration. One of these two thromboses is seen in the arteriole leading up to a glomerulus. One glomerulus is seen to contain a thrombus not associated with leukocytic reaction. Within some of the larger arteries of both cortex and medulla there are small masses of hyalinized fibrin covered by a thin layer of endothelial cells.

Urinary Bladder: The bladder shows the same changes that were found in the intestine, namely, thrombosis and perivascular leukocytic infiltration of the arterioles of the submucosa and muscularis.

Brain and Spinal Cord: A section of the lower thoracic spinal cord shows normal meninges, but within the fiber tracts are a few small, focal, perivascular cellular accumulations. There are two more such perivascular lesions at the extreme edge of one of the anterior horns; the cells are mainly neuroglia cells, but there are also some monocytes out in the tissue about the blood vessels (Fig. 4). Some of the vessels are thrombosed. A section of cerebral cortex shows a few polymorphonuclear and large mononuclear leukocytes within the pia-arachnoid (Fig. 12). Sections of cerebral cortex, optic thalami, pons, basal ganglia and olivary bodies show numerous examples of two apparently different types of lesions, and the sections of pons show a third type of lesion. The first type consists in the accumulation about blood vessels of large numbers of cells, predominantly neuroglial, although some monocytes are also present (Figs. 3 and 6). The endothelial cells of these vessels are swollen,

and some of the vessels are occluded, but many are not. The second type of lesion consists of occlusion of arterioles by polymorphonuclear and large mononuclear leukocytes, with infiltration of the surrounding tissue by some of these leukocytes (Fig. 5). The third type of lesion is a very striking one, and the lesions are much more extensive than the others. These are all in close association with partially or completely occluded arterioles, and consist in degeneration in an adjacent focus of the axones, which have become greatly swollen and nearly globular at points. Where the fiber tracts are cut transversely the appearance is that of large droplets (Fig. 2), but longitudinal section shows the "droplets" to be continuous with the fibers at each end (Fig. 1). Many of these globular swellings show a collection of very fine blue granules at one pole, and some show them at both poles. Occasionally one is seen which somewhat resembles a degenerated ganglion cell, but there cannot be the slightest doubt that practically all, if not all, of these structures are derived from axones. These structures show considerable variation in size, as reference to the plates will prove. Some large vacuoles are seen in the midst of these collections of degenerated axones, presumably resulting from complete degeneration and loss of the axonal material. That these lesions are areas of swelling of the axones is evidenced by the outward bowing of the normal fiber tracts which pass these areas and by the spaces at the edge of the lesions between the axones entering the lesions, as well as by the appearance of the lesions themselves. These lesions are less numerous than are the other two types, but are by no means rare. They appear to be miliary infarcts resulting from arterial thrombosis. In addition to the true perivascular cellular infiltration, many of the arteries within the brain tissue show an accumulation of monocytes in the stroma which accompanies them in their course (Fig. 13). Examples of this are most numerous in the pons, but are very abundant in the olivary bodies, basal ganglia and thalami.

Vertebral Bone Marrow: Not remarkable.

Skin: Sections of skin from the buttocks and thigh show small areas of hemorrhage in the corium, thrombosis of the capillaries and arterioles in the skin, and thrombosis of the large arteries in the subcutaneous fatty tissue. The walls of the vessels in the true corium show much damage and the media is infiltrated by monocytes and polymorphonuclear leukocytes. The tissue about the thrombosed

vessels contains many monocytes and polymorphonuclears, and a few plasma cells and mast cells. This perivascular leukocytic infiltration is most striking about the smaller arterioles and capillaries. Mast cells are seen in small numbers about the thrombosed vessels in other tissues, but are not as numerous as in the skin. Although the majority of the larger affected vessels in the subcutaneous fatty tissue are not completely thrombosed, many are, and many others which contain no thrombi are distended by blood. Some of the thrombosed vessels show hardly any leukocytic infiltration of their walls, but others have many leukocytes in their media and adventitia (Figs. 7 and 8). The thrombosed vessels are all arteries, the veins being apparently uninjured. Although no effort was made to secure serial sections, many sections from each block were examined and a satisfactory, although imperfect, approximation to a continuous series was thus obtained. In none of these sections is the perivascular leukocytic infiltration so widespread as to resemble the characteristic typhus nodules. The thrombi incorporate varying numbers of polymorphonuclear leukocytes and monocytes (endothelial leukocytes); in some thrombi the former predominate, and in others the latter are most abundant. Sections stained by Giemsa's method and by Goodpasture's carbol anilin fuchsin stain [9] show *Rickettsiae* within endothelial cells and muscle cells of the media of thrombosed arteries (Figs. 14 to 19).

Discussion

As justification for the diagnosis of Rocky Mountain spotted fever in this case several facts are offered. First, the history of tick bite followed by a disease which fits almost perfectly the picture of spotted fever described by Dyer and his coworkers, and which cannot be reconciled with their description of endemic typhus. Second, the rarity of typhus in children and the very low mortality rate of typhus also militate against the diagnosis of typhus. Third, the pathological identity of this case with those studied by Lillie. And fourth, experimental work by Dr. William Litterer, Director of the Division of Laboratories of the Tennessee State Health Department.[10] This work has not been completed, but offers conclusive immunological evidence that the child did not have typhus. Blood taken during the last days of the patient's illness was injected intra-

peritoneally into guinea pigs, and the strain of virus has been maintained to date by passage through guinea pigs. All of the animals given this disease have died; however, up till now only a relatively small number of animals has been used. Guinea pigs which have recovered from an attack of typhus induced by inoculation with a strain of endemic typhus virus maintained in the laboratory are not immune to the spotted fever virus from this case. The reciprocal cross immunity has not yet been done.

The lesions found in this case are almost identical with those described by Lillie, differing in only a few minor details. Thrombosis and perivascular leukocytic infiltration in the mucosa and submucosa of the gastro-intestinal tract was present in this case and absent in the one case of Lillie's in which the gastro-intestinal tract was examined. In our case a few arterioles in the lungs contained thrombi. This was not seen in Lillie's cases. The only important difference is in the renal lesions. In Lillie's cases there were many foci of perivascular lymphocytic infiltration; in our case the predominant cells in the perivascular accumulations were polymorphonuclear and large mononuclear leukocytes.

The brain lesions observed in this case are identical with those described and illustrated by Lillie, and with the exception of our third type (Figs. 1 and 2) are also identical with those noted by Wolbach [11] in his study of European typhus. This third type of lesion has not been seen in an incontrovertible case of typhus. It was apparently seen by Pinkerton and Maxcy [12] in a case diagnosed as endemic typhus, but it seems probable that their case was actually one of Rocky Mountain spotted fever instead.

This third type of brain lesion appears to be characteristic of the Eastern form of Rocky Mountain spotted fever, and may prove to be a reliable histological criterion for the differential diagnosis of the two diseases. The occurrence of brain lesions in the Eastern form of the disease is apparently constant and constitutes an important difference between the Eastern and Western forms. These points can be clarified only by the study of more brains from human cases of the two forms of this disease.

Demonstration of *Rickettsiae* in sections of skin was first accomplished with Giemsa's stain, using Wolbach's technique.[6] Since Goodpasture's carbol anilin fuchsin stain had been found to give such excellent results with other microörganisms, we wished to see

how well it would work with *Rickettsiae*. Wolbach tried this stain with the technique originally used and found that it gave fair results, but was confusing because of intense staining of other material occurring in the lesions. Goodpasture subsequently improved the technique,[9] and this modification was employed in the present study. If this technique is strictly followed it will be found that it will be necessary to run the sections through more than half a dozen times in order to stain the organisms deeply; for this reason the sections were stained for four minutes or more before decolorization, and running them through four times gave very intense staining of the organisms. If tissues other than skin are stained by this method they must not be stained for much more than a minute, for otherwise they cannot be sufficiently decolorized. This method gave very satisfactory results with the skin sections, the organisms within the muscle cells standing out strikingly, and all the photomicrographs of the organisms were made from sections stained by this method. For demonstration of the organisms within the muscle cells, and according to Wolbach they are found most abundantly in the smooth muscle cells of the media of vessels with the lesions, this method gives results fully equal to, and in our opinion superior, to the results obtained with Giemsa's stain. Furthermore, the sections may be stained in much less time (less than an hour). For demonstration of the organisms within endothelial cells it is not so satisfactory, for the reason given by Wolbach. However, search of several sections will hardly fail to show a few endothelial cells in thrombosed vessels in which there is no question as to the presence of the organisms. One familiar with the appearance of *Rickettsiae* and familiar with the staining method will find it an excellent stain, but it cannot be recommended as one to be used routinely to replace Giemsa's stain in the study of Rickettsial diseases, although it is a stain which may be used profitably to supplement Giemsa's method.

In addition to the two cases of Rocky Mountain spotted fever already mentioned in this paper, three other cases occurred in Tennessee in 1931. All five cases occurred within a relatively short distance of Nashville, and all the cases developed shortly after tick bite. In two cases the diagnosis was purely clinical, but the other three cases were shown by immunological tests not to be typhus. There was only one death in this series of cases.

Summary

1. The occurrence of Rocky Mountain spotted fever in Tennessee and a histological study of a fatal case are herewith reported.

2. The occurrence of brain lesions in the Eastern form of Rocky Mountain spotted fever reported by Lillie is confirmed.

3. Attention is called to a type of brain lesion which may prove to be diagnostic of the Eastern form of Rocky Mountain spotted fever.

REFERENCES

1. Wood, W. W. Spotted fever as reported from Idaho. Report of the Surgeon-General of the Army to the Secretary of War, 1896.

2. LaBier, C. R. Rocky Mountain spotted fever in Indiana. Report of a case. *J. Indiana M. A.*, 1925, **18**, 418–419.

3. Dyer, R. E., Rumreich, A. S., and Badger, L. F. The typhus-Rocky Mountain spotted fever group in the United States. *J. A. M. A.*, 1931, **97**, 589–594.

4. Rumreich, A., Dyer, R. E., and Badger, L. F. The typhus-Rocky Mountain spotted fever group. An epidemiological and clinical study in the Eastern and Southeastern states. *Pub. Health Rep.*, 1931, **46**, 470–480.

5. Badger, L. F., Dyer, R. E., and Rumreich, A. An infection of the Rocky Mountain spotted fever type. Identification in the eastern part of the United States. *Pub. Health Rep.*, 1931, **46**, 463–470.

6. Maxcy, K. F. Clinical observations on endemic typhus (Brill's Disease) in southern United States. *Pub. Health Rep.*, 1926, **41**, 1213–1220.

7. Wolbach, S. B. Studies on Rocky Mountain spotted fever. *J. Med. Res.*, 1919, **41**, 1–197.

8. Lillie, R. D. Pathology of the Eastern type of Rocky Mountain spotted fever. *Pub. Health Rep.*, 1931, **46**, 2840–2859.

9. Goodpasture, E. W. A study of rabies, with reference to a neural transmission of the virus in rabbits, and the structure and significance of Negri bodies. *Am. J. Path.*, 1925, **1**, 547–582.

10. Litterer, W. Personal communication to the author.

11. Wolbach, S. B., Todd, J. L., and Palfrey, F. W. The Etiology and Pathology of Typhus. Harvard University Press, Cambridge, Mass., 1922.

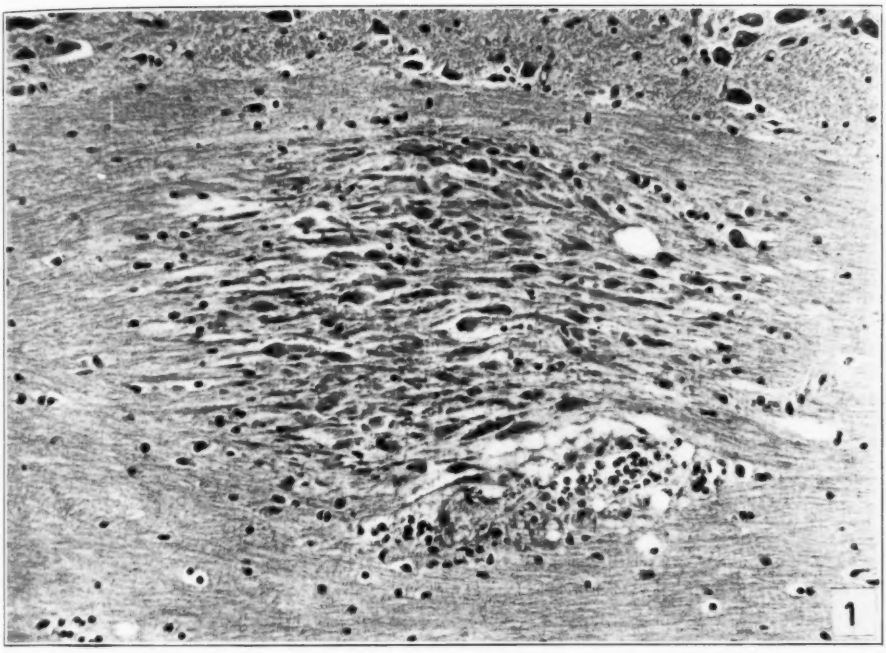
12. Pinkerton, H., and Maxcy, K. F. Pathological study of a case of endemic typhus in Virginia with demonstration of Rickettsia. *Am. J. Path.*, 1931, **7**, 95–104.

DESCRIPTION OF PLATES

PLATE 15

FIG. 1. Pons. Longitudinal section of focus of axonal degeneration. A thrombosed arteriole containing many polymorphonuclear and large mononuclear leukocytes is seen at the inferior edge of the lesion. Phosphotungstic acid hematoxylin stain. × 200.

FIG. 2. Pons. Cross-section of a similar area. A thrombosed arteriole is seen 2 cm. to the left of the middle of the photograph. Phosphotungstic acid hematoxylin stain. × 325.



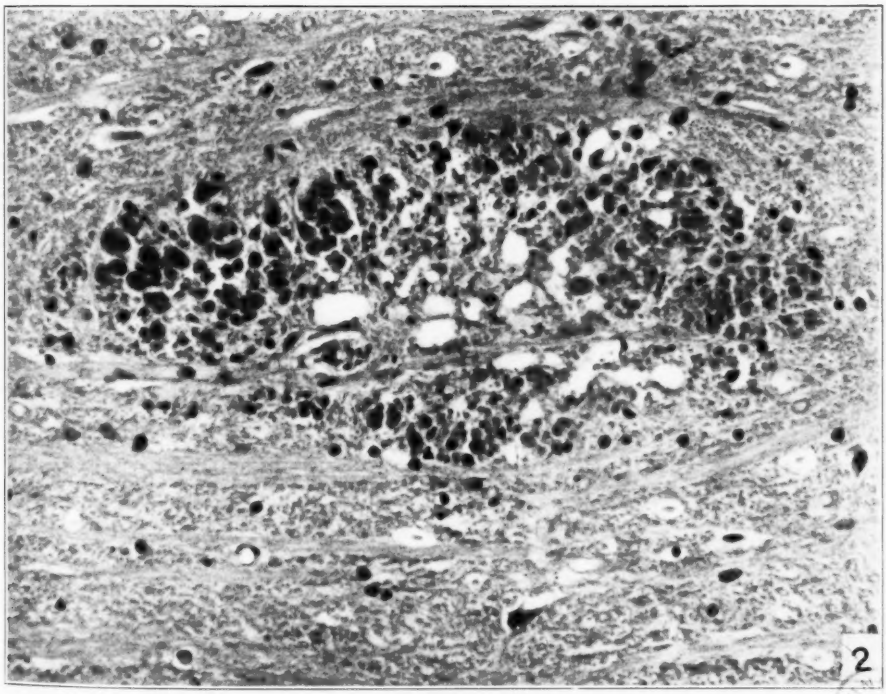


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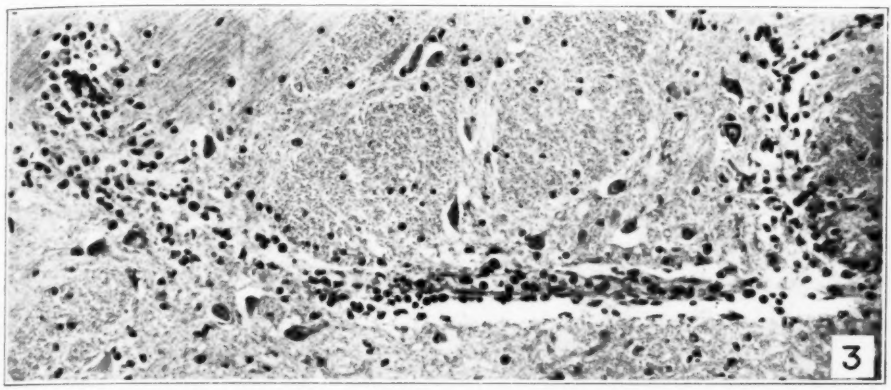
PLATE 16

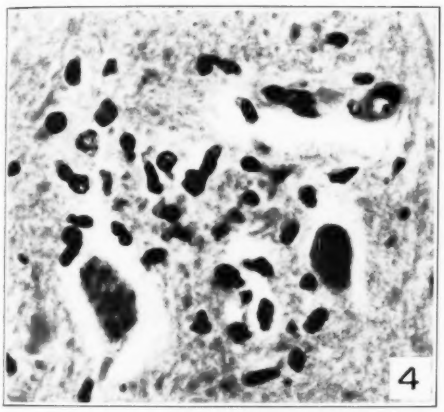
FIG. 3. Pons. This field shows thrombosis of an arteriole at the left, perivascular cellular infiltration, and leukocytic infiltration of the pia about the blood vessel. Phosphotungstic acid hematoxylin stain. × 200.

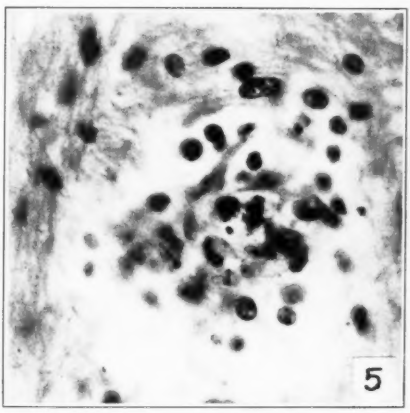
FIG. 4. Spinal Cord. Perivascular lesion with glia cells and some large mononuclear phagoctyes. Hematoxylin and eosin stain. × 350.

FIG. 5. Pons. Thrombosed arteriole with polymorphonuclear and large mononuclear leukocytes. Note cellular débris within the leukocytes. Phosphotungstic acid hematoxylin stain. × 550.

FIG. 6. Pons. Perivascular lesion of typhus type. Phosphotungstic acid hematoxylin stain. × 550.







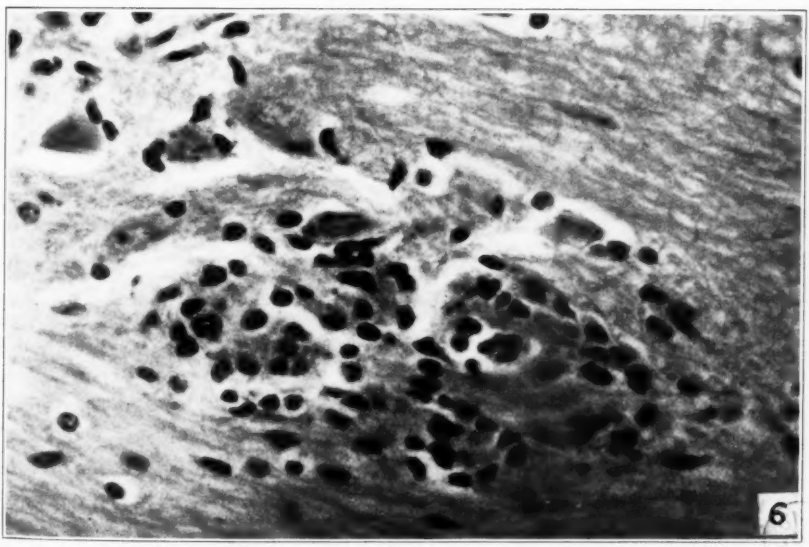


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PLATE 17

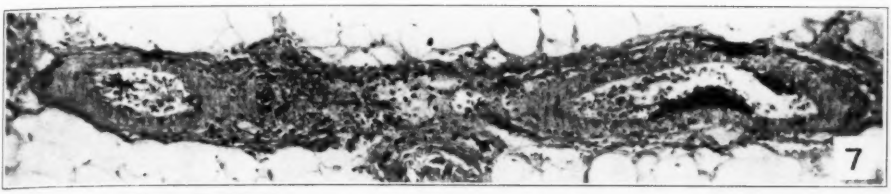
FIG. 7. Artery in subcutaneous fatty tissue. Note thrombosis, extensive leukocytic invasion of the media, and very slight perivascular infiltration. × 100.

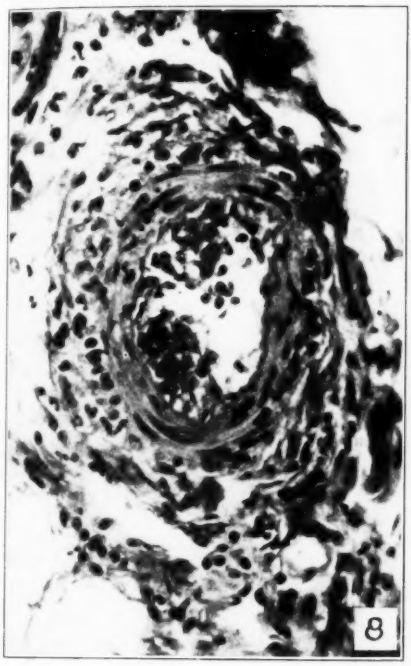
FIG. 8. Artery in subcutaneous fatty tissue. Note thrombosis and leukocytic invasion of the vessel wall. There is far more leukocytic invasion of the tissues about the vessel than is usually seen. Hematoxylin and eosin stain. × 325.

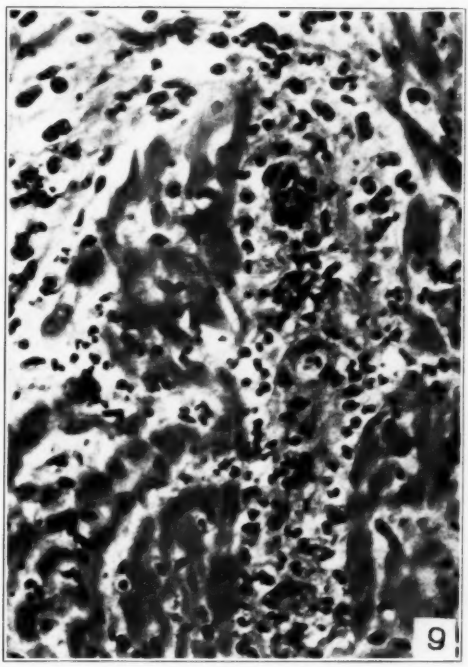
FIG. 9. Heart. In addition to the marked interstitial leukocytic infiltration several thrombosed arterioles and capillaries are seen. Hematoxylin and eosin stain. × 375.

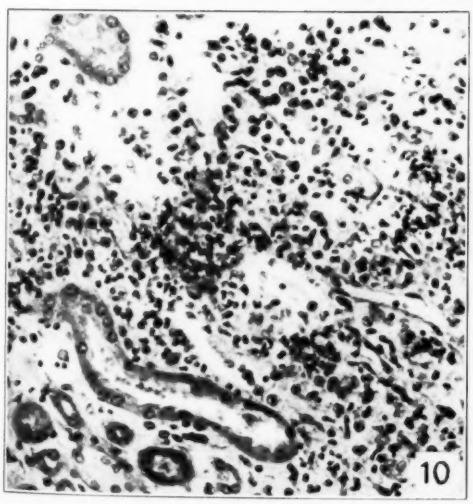
FIG. 10. Kidney. One of the larger lesions is shown here. There is extensive leukocytic invasion with necrosis of tubules. Two thrombosed vessels are seen, one near the middle of the photograph, and the other half way between this and the lower right corner. Hematoxylin and eosin stain. × 225.

FIG. 11. Kidney. One of the smaller lesions. Note the thrombosed vessel with extensive leukocytic infiltration of the interstitial tissue and absence of damage to the tubules. Hematoxylin and eosin stain. × 325.









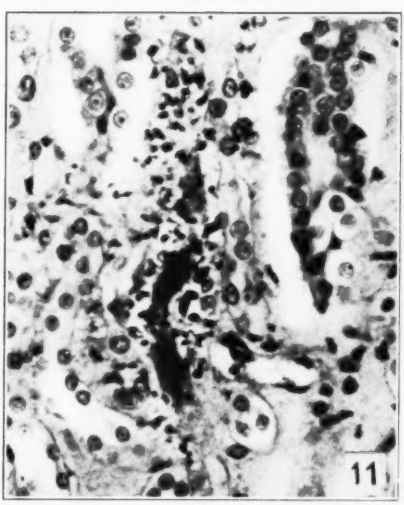


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PLATE 18

FIG. 12. Cerebral meninges. This photograph shows clearly the types of cells appearing in the exudate. Hematoxylin and eosin stain. × 250.

FIG. 13. Artery in region of olivary bodies. This shows the type of cell infiltrating the pial vestment of the arteries. Hematoxylin and eosin stain. × 375.

FIG. 14. Group of *Rickettsiae* within muscle cell of media. A definite halo may be seen about most of the organisms. A portion of thrombus is seen at the lower left.

FIG. 15. Two groups of *Rickettsiae* within two different muscle cells.

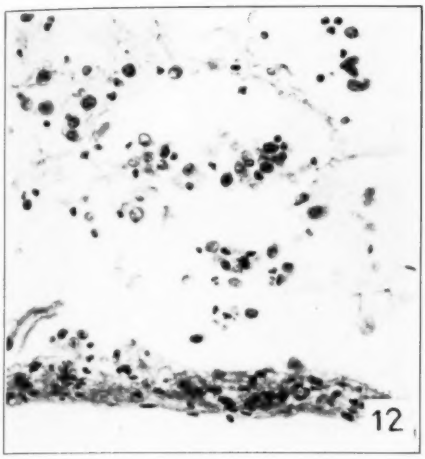
FIG. 16. Group of *Rickettsiae* within a muscle cell. A portion of thrombus is seen below.

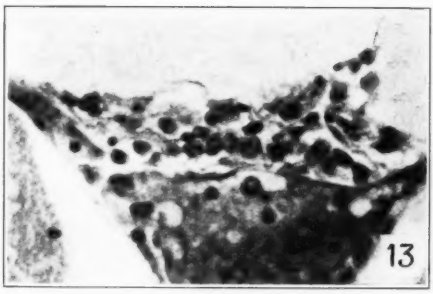
FIG. 17. The same field seen immediately above in Fig. 15, but focused at a slightly different level to show groups of *Rickettsiae* within adjacent muscle cells.

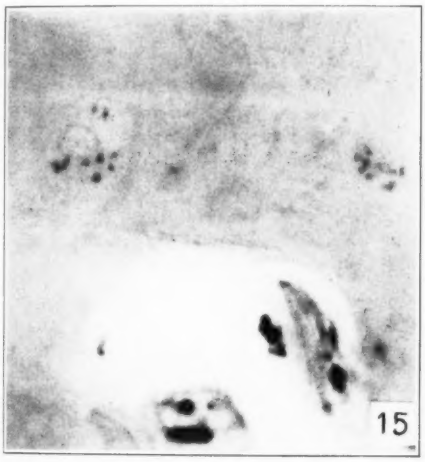
FIG. 18. Endothelial cell containing two groups of *Rickettsiae*, one above the nucleus, and one below. Those above the nucleus are surrounded by a very distinct halo.

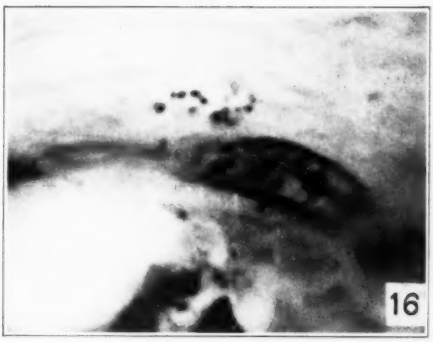
FIG. 19. Two groups of *Rickettsiae*. One group within an endothelial cell below the nucleus, and the other group within a muscle cell to the left of the endothelial cell nucleus. Several of these organisms are surrounded by a faint halo.

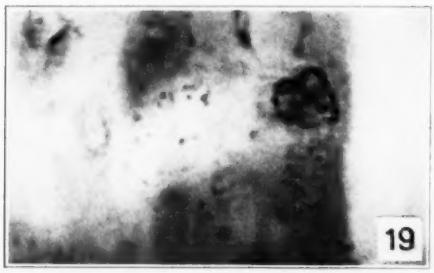
(FIGS. 14 to 19 are photomicrographs of *Rickettsiae* found in the walls of thrombosed arteries in the skin. All were stained by Goodpasture's carbol anilin fuchsin stain and are magnified 2700 diameters, except Fig. 19 which is magnified only 2000 diameters.)











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Eastern Type of Rocky Mountain Spotted Fever

METASTATIC CALCIFICATION OCCURRING IN MYELOGENOUS LEUKEMIA *

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Metastatic calcification is a term originally employed by Virchow to designate a condition in which there is mobilization of the body calcium from its primary depots in the bones and redistribution to other tissues and organs of the body. Virchow's original inferences that there was oversaturation of the blood with calcium derived from the bones and redeposition in distant sites of the body found universal acceptance because the condition usually accompanied disease in which there was extensive bone destruction, such as osteomyelitis, tuberculosis of bone, primary and metastatic tumors, and leukemias.

Wells[1] reviewed the subject of metastatic calcification, and earlier[2] compared the processes of calcification and ossification. He reported a case of myelogenous leukemia in which calcium metastasis occurred and quoted the experiments of Tanaka[3] and others, who reproduced the condition in animals after intraperitoneal injections of calcium salts. In a careful review of the literature he discovered twenty-nine cases of this condition, to which he appended one of his own. The following, it is believed, is the second to be recorded in the American literature.

REPORT OF CASE

Clinical History: The patient, F. L., a girl 19 years of age, unmarried, native born, was admitted to the Fourth Medical Division of Bellevue Hospital on Dec. 5, 1931. Her chief complaints were weakness and general malaise, loss of appetite and weight, and enlargement of the abdomen of a year's duration. The family history was essentially negative. As a child the patient had always been frail and of delicate health, but had had only the more common childhood diseases such as measles and diphtheria. On Feb. 24, 1931, she had been admitted to another hospital complaining of an itching rash of two weeks' duration.

* Received for publication July 25, 1932.

At that time the spleen was enlarged and the blood picture was characteristic of myelogenous leukemia.

Physical Examination: Patient appeared as a frail white girl, extremely anemic, underweight and chronically ill. The skin and mucous membranes were pale, the teeth in good repair, the tonsils small. The neck showed a small discrete adenopathy of the angular and supraclavicular lymph nodes. The heart and lungs were essentially normal. A large mass, undoubtedly spleen, occupied the entire left half of the abdomen from the xyphoid process to the crest of the ileum. This was not tender. The liver was enlarged to two fingers' breadth below the costal margin. Extremities showed muscular wasting but no edema. Small, discrete, bilateral inguinal lymphadenopathy was present.

Laboratory Findings: The blood pressure remained about 100/60 throughout the course of illness. The urine always showed considerable albumin and many white blood cells. Wassermann test negative. Blood total nitrogen 30, sugar 79 mg. per 100 cc. Basal metabolism on December 15 was + 75. On admission total white blood count was 21,200, 63 per cent immature forms, 22 per cent adult polynuclear neutrophiles, 2 per cent eosinophiles, 13 per cent lymphocytes, and the red blood count 2,200,000.

Course of Illness: On December 7 sodium cacodylate, 1 gm. daily was started.

On December 8 the blood showed 38 per cent hemoglobin, 3,080,000 red blood cells, 37,000 white blood cells. Smear showed many normoblasts and a predominance of young granulocytic forms.

December 16 the patient was very weak and complained bitterly of abdominal and lumbar pain which required large and frequent dosages of codein. The blood picture remained essentially unchanged.

December 19 sodium cacodylate was discontinued because the patient had developed a garlic odor to the breath. Dullness and moist râles were present at the base of left lung.

December 22 the patient received a transfusion of 500 cc. of whole blood.

On December 24 the total white blood count rose to 66,000.

December 29 radiation therapy was started. This was repeated once on December 31. At this time there was pronounced mental lethargy.

On Jan. 1, 1932, the patient was extremely toxic and comatose. She presented a picture of terminal failure characterized by embryocardia, diffuse chest râles and coma. A blood count just before death showed 1,200,000 white blood cells, of which 92 per cent were granulocytes.

Death occurred at 6.00 A.M. Jan. 2, 1932.

Autopsy Report

Autopsy was performed three hours after the patient's death. The anatomical diagnoses were: myelogenous leukemia with involvement of the spleen, liver, kidneys, retroperitoneal and mediastinal lymph nodes, and the superficial supraclavicular and axillary lymph nodes; metastatic calcification of the left auricle, pulmonary veins, aorta and main pulmonary artery, and of the renal, coeliac, mesenteric and orifices of the coronary arteries.

The body was that of a young, adult, white female, who appeared about 15 years of age, of delicate slender frame, moderately emaciated. Body length 158 cm., weight 95 lbs. Skin smooth, pale and white. Nodular masses about $1\frac{1}{4}$ cm. in diameter were palpable in both supraclavicular fossae and in the axillae.

Subcutaneous fat was absent over the thorax and slight over the abdomen. Recti muscles were delicate and poorly preserved. The spleen occupied the entire left side of the abdominal cavity from the dome of the diaphragm above to the pubic symphysis, displacing the small intestines to the right side. The superior anterior surface of the spleen was adherent to the posterior surface of the left lobe of the liver. The transverse colon dipped posteriorly to the upper half of the spleen, which appeared to compress it at that point.

The thoracic cavity was essentially normal.

Heart: Weight 250 gm. Right auricle and ventricle contained large masses of chicken-fat clot. Left auricle contained similar clot. Its endocardial surface was raised, thickened and had a chalky white color. The valves were normal. The endocardium in the sinuses of Valsalva of both pulmonary and aortic valves was thrown into a series of parallel longitudinal ridges about 1 mm. in length. These ridges occurred in the mouths of the coronary arteries. The entire length of the aorta showed at regular intervals a succession of these parallel ridges. The various visceral arteries, splenic, coeliac, mesenteric and renal, were thrown up into these same ridges which felt brittle and often involved the entire cross-section of the vessel.

Lungs: Right lung weighed 750 gm., the left lung 650 gm. The visceral pleura was pale pink and glistening. The lungs were generally crepitant, save for areas in the periphery where small scattered infarcts were present. When sectioned, a grating sensation was imparted to the knife. The vessels were gray, felt calcified and projected a few millimeters from the cut surface. The apertures were frequently thrombosed and often gaping.

Liver: Greatly enlarged, weighing 2750 gm., and measuring 35 by 22.5 by 15 cm. The color was dark reddish brown. Capsule smooth, edges sharply defined. On section consistence slightly increased. Small, linear, pale areas were visible throughout the cut surface, especially beneath the capsule.

Spleen: Greatly enlarged, weighing 2550 gm., measuring 37.5 by 15 by 10 cm. Splenic artery showed calcific changes as described.

There were two shallow notches at the mesial border and a large, solitary, wedge-shaped infarct just beneath the hilum. On section consistence firm, surface red and coarsely granular.

Kidneys: Combined weight 400 gm. The capsule stripped readily, surface smooth, shiny and the color of chicken-fat. On section cortex slightly swollen and the cortical markings practically obliterated.

Lymphatics: The perigastric, posterior mediastinal, superior and inferior periaortic and iliac nodes, as well as the superficial groups, were enlarged. The retroperitoneal nodes varied from 1 to 6 cm. in diameter, and were usually discrete, save for the periaortic groups which were fused. Cut surface had the appearance of fish meat.

Microscopic Examination

Tissues from the various organs were removed and fixed for 48 hours in 10 per cent formol solution, after which sections were embedded in paraffin and stained with hematoxylin and eosin, by Giemsa's method and with Weigert's elastic tissue stain. Smears of the bone marrow were stained with Wright's blood stain.

The bone marrow smears show fairly typical myeloid transformation with every variety of granulocyte present, particularly the early forms.

The liver, spleen and lymph nodes show characteristic leukemic deposits.

The endocardium of the left auricle is the site of extensive calcium deposition which is sharply limited to the subendothelial elastic fibers. These are swollen, often ruptured and impregnated with calcium salts, assuming an intense purple stain with hematoxylin. The elastic fibers are separated by numerous necrotic cells, probably leukocytes, themselves staining deeply for calcium. The deeper layers contain numerous neutrophilic leukocytes spreading apart the auricular myofibrils. The vasa vasorum of the myocardium and epicardium contain leukemic thrombi.

The arterial changes are best demonstrated in the aorta. Hematoxylin and eosin-stained sections demonstrate that a selective calcification is confined almost exclusively to the inner elastic lamina, which like the elastic fibers of the auricle described above, is swollen, stains deeply with hematoxylin and is thrown into exaggerated folds corresponding to the macroscopic ridges. With Weigert's elastic

tissue stain some of the elastic fibrils are seen to be ruptured. Sections of the main pulmonary artery and of the smaller visceral arteries, particularly the splenic, reveal identical changes. In every case the grossly visible ridges in the vessel walls are seen to correspond to points of rupture of the calcified elastic laminae.

Lung sections reveal a uniform, widespread and pronounced dilatation of the pulmonary veins by leukemic thrombi. In sections these distended vessels are so prominent as to comprise almost one-fifth of the area of a cross-section. The intima of these veins shows widespread calcification and fragmentation. Occasionally the calcified endothelium and subendothelial tissue is desquamated or partially detached from the vein wall. Where points of rupture of the elastica are present, the veins are thrown into bizarre patterns. A few arterioles show similar changes but the venous changes are predominant.

The interalveolar capillaries are distended with leukemic blood. Oftentimes the interalveolar septa are calcified and ruptured. No calcium is found deposited in the bronchial epithelium or cartilage.

The kidneys show leukemic infiltration of both cortex and medulla. Many of the tubules and ducts are converted into deep purple-staining masses of calcium. Often rings of calcium occupy the lumen. Elsewhere the lining cells show calcification of the intracellular substances and detachment from the basement membrane. Occasionally, calcium is found in the glomerular tufts and in the space between capsule and tuft epithelium. The medullary ducts contain numerous leukocytes, many of which stain for calcium.

Discussion

The lesions described above are fairly typical of those to which the term "metastatic calcification" has been applied. Metastatic calcification differs from a number of types of pathological calcification in that previous degenerations or necroses of tissue are not essential prerequisites. It is best regarded as a fundamental disturbance of calcium equilibrium in which calcium is mobilized from the depots in the long bones and reprecipitated in other organs and tissues of the body.

As Wells pointed out, the elastic tissues of the body, especially the elastic laminae of systemic arteries, are favorite sites for calcium

metastases. Wells believes that this predeposition of the elastic tissues resides in the physical constitution of elastic fibrils, which under certain conditions are capable of adsorbing soluble calcium salts and reprecipitating them. Change of alkalinity of the tissue fluids probably favors precipitation. He pointed out, too, that those organs which most frequently show this phenomenon generally excrete acids, whereupon there is a decrease in the acidity of the remaining tissue fluids — a reaction favoring precipitation of calcium salts. Thus, the lungs excrete carbonic acid, the gastric mucosa hydrochloric acid, the kidneys acid phosphates. Hofmeister [4] showed that the occurrence of metastatic calcification in the gastric mucosa was limited to interglandular spaces in the region of the fundus where the acid-secreting glands are located.

In the lungs where CO_2 is excreted, the calcification, as would be expected, occurs in the alveolar septa and in the veins. In the left auricle and in the peripheral blood vessels where the CO_2 tension of the blood is diminished, metastatic calcification is also found.

Where metastatic calcification has been encountered it has invariably occurred in bone diseases, primary and secondary neoplasms, suppurative processes and in leukemias. All of these conditions are characterized in common by osteolytic bone changes. Osteolysis, while it is uncommon, does occur in leukemias. Craver and Copeland [5] have recently made roentgenographic studies of the bone changes in leukemias. Of 100 cases of lymphatic leukemia and 83 cases of myelogenous leukemia analyzed by them, 3 of the former and 1 of the latter showed definite roentgenographic evidence of bone involvement. The X-ray picture usually showed cortical destruction with punched-out areas of osteoporosis. The bones found most frequently involved were usually the pelvis, humerus, ulna, radius, spine and skull. The infrequency of these osseous lesions in the leukemias would correspond to the rarity of metastatic calcification as a postmortem finding.

Conditions indistinguishable from metastatic calcification have recently been reproduced in experimental animals by injection of parathormone (Collip) [6, 7] and by the prolonged ingestion [8] of radiated ergosterol. Both these substances elevate the concentration of blood calcium. The source of the increased blood calcium following parathormone injections is undoubtedly the long bones, as has been shown by Bauer, Aub, and Albright,[9, 10] who found that loss

of trabeculae in the long bones of rabbits resulted after successive increasing dosages. That parathormone is capable of elevating blood calcium, even on a calcium-free diet, has been demonstrated by Hess and Lewis [11] and others. A marked hypercalcemia results when these animals are fed a normal diet, however. The mechanism whereby ergosterol and its allies produce hypercalcemia and metastatic calcification is unknown, though the fact that these substances may stimulate the parathyroid glands has been advanced as a likely explanation of their action.

The writer has observed calcification of the pulmonary alveolar septa indistinguishable from that in the above case in a patient with chronic diffuse glomerulonephritis who died in uremia. Wells quotes Schmidt [12] who described calcification of the myocardium of the left ventricle, pericardium, lungs, kidneys, pulmonary veins, stomach and systemic arteries in a patient with advanced nephritis. It appears not unlikely that such forms of calcification are the same as those of the metastatic variety.

Summary

1. A case is reported of metastatic calcification occurring in a young girl whose history indicated that she had had myelogenous leukemia for at least two years.

2. The term is employed to designate a condition in which calcium is mobilized from the depots in the bones and reprecipitated in other tissues of the body, particularly elastic tissues, which are probably physically constituted to favor calcium adsorption and reprecipitation. Calcium deposition is also seen to be favored by any phenomenon which reduces the acidity of the tissue fluid. Hence, metastatic calcification occurs in those tissues where a change in the alkaline direction very likely occurs.

3. Recent evidences mentioned show that the leukemias are rarely associated with osteolytic changes in the skeleton and this infrequency probably explains the rarity of metastatic calcification as a finding in leukemias.

4. Finally, the identity of this condition with the calcifications occurring after administration of parathormone and irradiated ergosterol is reaffirmed.

REFERENCES

1. Wells, H. G. Metastatic calcification. *Arch. Int. Med.*, 1915, **15**, 574.
2. Wells, H. G. Calcification and ossification. *Arch. Int. Med.*, 1911, **7**, 721.
3. Tanaka, M. Über Kalkresorption und Verkalkung. *Biochem. Ztschr.*, 1911, **35**, 113.
4. Hofmeister, F. Über Ablagerung und Resorption von Kalksalzen in den Geweben. *Ergebn. d. Physiol.*, 1910, **10**, 429.
5. Craver, L. F., and Copeland, M. M. Quoted by Geschickter, C. F., and Copeland, M. M. Tumors of Bone. Amer. J. Cancer, pub. N. Y., 1931, 617.
6. Hueper, W. Metastatic calcifications in the organs of the dog after injections of parathyroid extract. *Arch. Path. & Lab. Med.*, 1927, **3**, 14.
7. Learner, A. Calcium deposition in tissues of dogs and mice by the aid of parathormone. *J. Lab. & Clin. Med.*, 1928–29, **14**, 921.
8. Demole, V., and Fromherz, K. Serumcalcium und Organverkalkungen unter der wirkung von besstrahltem Ergosterin. *Arch. f. exper. Path. u. Pharmakol.*, 1929, **146**, 347.
9. Bauer, W., Aub, J. C., and Albright, F. Studies of calcium and phosphorus metabolism. A study of the bone trabeculae as a readily available reserve supply of calcium. *J. Exper. Med.*, 1929, **49**, 145.
10. Bauer, W., Albright, F., and Aub, J. C. A case of osteitis fibrosa cystica (osteomalacia?) with evidence of hyperactivity of the parathyroid bodies. Metabolic study II. *J. Clin. Investigation*, 1929–30, **8**, 229.
11. Hess, A. F., and Lewis, J. M. Clinical experience with irradiated ergosterol. *J.A.M.A.*, 1928, **91**, 783.
12. Schmidt, M. B. Kalkmetastase und Kalkgicht. *Deutsche med. Wchnschr.*, 1913, **39**, 59.

DESCRIPTION OF PLATE

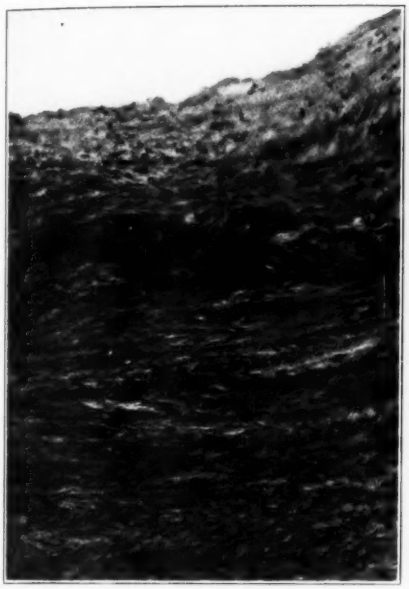
PLATE 19

FIG. 1. Photomicrograph of aorta showing selective calcification of the inner elastic lamina. Hematoxylin-eosin stain. × 100.

FIG. 2. Photomicrograph of left auricle. Note the leukemic infiltration. The dense black areas represent areas of calcified elastic tissue fibrils. Hematoxylin-eosin stain. × 120.

FIG. 3. Photomicrograph of lung. Note the dense calcification of the pulmonary veins and of the interalveolar septa. Numerous points of rupture of the calcified elastica are seen. Weigert's elastic tissue stain. × 100.

FIG. 4. Photomicrograph of kidney showing leukemic infiltration and calcification of some of the tubules. Hematoxylin-eosin stain. × 120.



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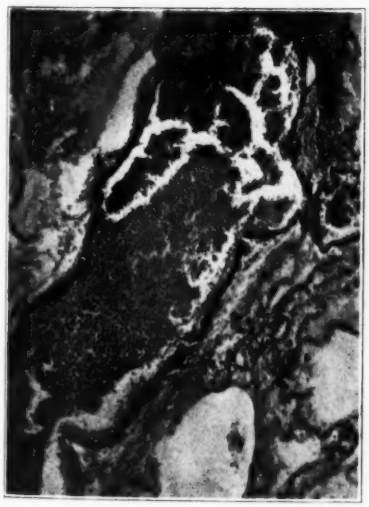

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DeSanto

Metastatic Calcification in Myelogenous Leukemia

MYELOBLASTIC SARCOMA OF THE SCAPULA ASSOCIATED WITH CHRONIC SPLENOMYELOGENOUS LEUKEMIA *

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Hyperplasia of the hemopoietic tissues of bone marrow in myelogenous leukemia has prompted many speculative considerations of the disease. Some propose that leukemias are tumors, but the arguments favoring or contradicting this interpretation of the disease are indecisive. A patient after many years illness with chronic myelogenous leukemia occasionally has a growth of hemopoietic tissues in a bone and, because of local destructive extension and metastases into the viscera, the neoplasm has the characteristics of a malignant, marrow tissue tumor. Such conditions are described in this report. The myelogenous leukemia had been recognized for four years. The destructive tumor of the left scapula, and metastases in the axillary, biliary and cervical lymph nodes, the liver, pleura, spleen and twelfth thoracic vertebra, were demonstrated by the postmortem examination.

REPORT OF CASE

Clinical History: A white woman, aged 58 years, was in the care of Dr. Ralph C. Larrabee, Boston, from October 1927 until January 1930 for chronic splenomyelogenous leukemia. When first examined by Dr. Larrabee the patient's blood contained 2,792,000 erythrocytes, 312,800 leukocytes, 496,000 platelets per cmm. and the hemoglobin was 52 per cent. The distribution of the leukocytes was as follows: 55 per cent neutrophilic polymorphonuclear leukocytes, 5 per cent lymphocytes, 2 per cent mast cells, 37 per cent neutrophilic and 1 per cent eosinophilic myelocytes. The patient reacted well to roentgen therapy over the spleen and on Dec. 19, 1927, the hemoglobin was 74 per cent and the leukocytes 18,600 per cmm. of blood. Evidence of leukemia was almost absent except for the persistence of many mast cells. During the spring of 1928, the leukemic condition of the blood returned and the patient became refractory to roentgen therapy. By October 1928, the hemoglobin was 44 per cent, the red cells 2,312,000 and the white cells 400,600 per cmm. Of the leukocytes 40 per cent were neutrophilic and 1 per cent eosinophilic polymorphonuclear leukocytes, 4 per cent were mast cells, and 48 per cent neutrophilic, 2 per cent eosino-

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philic and 3 per cent mast cell myelocytes. Although three roentgen treatments over the spleen were ineffective, three over the long bones (tibiae) caused marked improvement. By October 1929, she had again relapsed and roentgen therapy was not effective. Her general condition was unfavorable.

In January 1930, she came under the care of Dr. Joseph A. Capps, Chicago, Ill. The blood had about 1,000,000 each of white and red blood cells per cmm. and the hemoglobin was 20 per cent. The spleen was especially large. At St. Luke's Hospital roentgen radiations of the chest dorsally were continued twice monthly during the winter and summer of 1930, and the patient's condition improved. The spleen decreased to about one-half its former size and on March 31, 1930, the blood elements were 3,200,000 erythrocytes, 49,000 leukocytes, and 61 per cent hemoglobin. Later the leukocyte count varied between 78,000 and 117,600, which it attained in September 1930. The following month the patient's physician in Boston found her spleen not enlarged, and the blood with 4,040,000 erythrocytes, 8400 leukocytes per cmm., and the hemoglobin 75 per cent. In the differential count of 100 leukocytes, 1 myelocyte, 10 eosinophils and 11 mast cells were the only abnormal cells found. The patient had gained considerable weight and felt well.

During the night of Jan. 26, 1931, she awoke with a severe pain in her back. The following day the lumbar spine was stiff to motion in all directions. Although there was no tenderness, an infarct of the spleen was considered probable. Subsequently there were several attacks of pain in the left shoulder, notably one on March 18, 1931, severe enough to require opiates for relief. On June 8 of that year, because of continued distress, X-ray pictures were taken of the left shoulder by Dr. George W. Holmes at the Massachusetts General Hospital. He noted moderate atrophy of the bones about the joint, but no evidence of tumor. On July 19, 1931, at the age of 62 years, she entered St. Luke's Hospital,Chicago, because of extreme general weakness and occasional recurrences of the pain in the left arm and shoulder. Emaciation, a huge spleen and a rough systolic murmur over the cardiac apex were the essentials of the physical examination.

The blood contained 2,290,000 erythrocytes, 18,900 leukocytes per cmm., and the hemoglobin was 39 per cent. Among 100 leukocytes there were 2 lymphocytes, 12 neutrophils, 3 eosinophils, 20 band-shaped polymorphonuclear leukocytes, 46 myelocytes, 13 metamyelocytes and 4 myeloblasts. The white cells continued to increase gradually to 42,000 per cmm., but dropped to 26,350 on August 17. The chief complaint was continuous pain in the left arm and occasionally in the left shoulder. On August 9 these tissues became swollen, indurated and tender, and a few small pale nodules appeared along the inner bicipital groove of the left arm. The temperature had ranged from 99 to 102,° but on August 19 it rose to 104.2° F, the pulse became 110 to 120 and the respirations 30 per minute. Death occurred Aug. 21, 1931, at 9.50 A.M.

Postmortem Examination

The essentials of the postmortem examination made an hour after death by Dr. Edwin F. Hirsch were: marked hyperplasia of the spleen (myelogenous leukemia); sarcoma of the left scapula; metastatic sarcoma of the left axillary lymph nodes, the biliary lymph nodes, the spleen, liver, pleura and twelfth thoracic vertebra;

marked calcified senile arteriosclerosis of the aorta and its main branches; moderate cloudy swelling and fatty change of the myocardium; senile arteriosclerotic atrophy of the kidneys; edema and hyperemia of the leptomeninges; slight hydropericardium, and organizing fibrinous perisplenitis.

The body weighed 113 pounds (50.4 kg.) and was 158 cm. long. Distal and lateral to the greater tubercle of the left humerus was a firm, freely movable, subcutaneous mass 2.5 cm. in diameter and elevated 2 cm. above the surrounding surface. This nodule was composed of dense white tissue lying close to the humerus between the two heads of the biceps brachii muscle. Tissues from this extended beneath the clavicle and dorsally to the subscapular fossa of the scapula, with which bone it was continuous. There was a marked destruction and roughening of the acromion process, spine, and glenoid fossa of the scapula. Continuous with this mass were firm, gray, enlarged axillary lymph nodes which likewise extended proximally into the lower left cervical lymph nodes. When completely dissected, the tumor tissue in the left axilla and anterior to the left scapula was roughly pyramidal in shape with the base 10 by 9 cm., resting on the left ribs and extending laterally 9.5 cm. The three faces of the pyramid were respectively 9 by 9.5 cm., 6 by 9.5 cm., and 8 by 9.5 cm. The surfaces made by cutting the tumor were mixtures of grayish pink and yellow necrotic tissues. The inguinal, right axillary and right cervical lymph nodes were not enlarged. The spleen measured 26 by 27 by 9.5 cm., and weighed 1620 gm. In and beneath the capsule on the diaphragmatic surface were many gray nodules with rounded surfaces; some as large as 2 cm. in diameter were in the firm, dark red, splenic parenchyma. The liver weighed 2170 gm. and had a smooth glistening capsule, except in the center of the right lobe where there was a glistening white nodule 4 by 2 mm. On surfaces made by cutting the liver there were many similar, small, indistinct white masses, and in the right lobe were four with diameters as much as 1 cm. Near the front and medial to the gall-bladder notch was a nodule 4 cm. in diameter with scalloped margins. On the right side of the body of the twelfth thoracic vertebra and continuous with the roughened bone was a nodule 1.7 by 1.5 by 1 cm. Deeply embedded in dense fibrous adhesions between the right lung and the diaphragm was a firm white nodule 1.2 by 1.3 cm. in its maximum dimensions.

The routine examination of the tissues included the lungs, innominate veins, superior and inferior venae cavae and their main branches, heart, aorta and its main branches, thoracic duct, thyroid gland, parathyroid glands, kidneys and ureters, pancreas, stomach, bowel, vagina, uterus, tubes, ovaries, urinary bladder, mesenteric lymph nodes, rectum, spine, ribs, and the bones.

Microscopic Examination

Blocks of the tissues for microscopic examination were fixed in Zenker's solution. Thin paraffin sections were stained with hematoxylin and eosin, Mallory's phosphotungstic acid hematoxylin,[1] eosin-methylene blue, Azur II-eosin and hematoxylin as used by Maximow,[2] and anilin blue azan (Heidenhain's modification[3]). Other blocks were fixed in formalin, sectioned by the freezing method and stained for oxydase granules, according to Graham's benzidine technique (Mallory and Wright,[1] see page 524) and for lipins with Sudan III.

In the primary tumor, a scanty fibrillar stroma supports compactly and also loosely arranged spherical cells 10 to 20 microns in diameter with large oval or round vesicular nuclei (Fig. 1). The narrow margins of cytoplasm contain fine oxydase granules. Many of these cells are like the hemacytoblasts of Maximow.[4] Elliptical cells 20 to 40 microns in diameter are intermingled with these. They have a scanty homogeneous or finely granular cytoplasm and two to six irregular vesicular nuclei. The distinction of nuclei is uncertain because of the marked lobulations. A few cells in mitosis are noted. Lipins are not present in their cytoplasm. These giant cells closely resemble megakaryocytes but differ in being smaller and in having larger vesicular nuclei and only a little cytoplasm. Many with few nuclei resemble the young megakaryocytes described and illustrated by Maximow[4] (see pages 116 and 118). A third variety of cell, intermediate in size and 20 to 25 mm. in diameter, has indistinct margins and contains a round, oval or indented nucleus. There is no bone production.

The distended sinusoids of the hyperemic hepatic tissues contain many leukocytes in various stages of development, and a few erythrocytes. There are fatty changes of the cells toward the central portions of the lobules. The metastatic tumor tissues have a loosely

arranged and edematous fibrous stroma which supports round, oval or polyhedral cells 10 to 25 microns in diameter with granular cytoplasm and vesicular nuclei (Figs. 2 and 3a). There are many giant cells as much as 60 microns in diameter with five or more oval or indented nuclei. The nuclei of others are fused lobed masses. Many cells are in mitosis (Fig. 3, e, g, h). These giant cells are similar to those in the primary tumor except that they are larger, more frequently in mitosis, and often appear to be syncytial masses such as might have been made by a fusion of smaller tumor cells. There are transitional forms between mononucleated and multinucleated tumor cells (Fig. 3, a, b, c, e, f). The scanty cytoplasm contains oxydase and neutrophilic and eosinophilic Ehrlich granules (Fig. 3d). These tumor cells in the liver metastases vary more in size, shape and staining reaction than those in the primary growth (Fig. 2).

Most of the cells in the nodule in the region of the twelfth thoracic vertebra are 10 to 20 microns in diameter with round or elliptical vesicular nuclei. Each contains a single nucleolus, and is surrounded by a scanty homogeneous cytoplasm. Intermingled with them are oval or polyhedral cells 15 to 20 microns in diameter with basophilic, eccentric, elliptical or semilunar nuclei, sometimes multiple. Giant cells, similar to those described elsewhere, are scattered in the tissues.

Follicles are indistinguishable in the hyperplastic, lower left cervical lymph node tissues. The lymphoid cells (largely plasma cells) are in small groups and columns separated and intermingled with polynuclear leukocytes in various stages of development. Tumor cells, like those previously described, are focally disseminated among the lymphoid elements. In one region the fine fibrillar stroma is in interlacing bands that form alveoli filled with tumor cells. Neutrophilic and eosinophilic granules are demonstrated in the cytoplasm of the tumor and myeloid cells.

In a lymph node from the region of the left biceps brachii, the fine fibrous stroma supports a few lymphocytes and many myelocytes. The latter fill the sinusoids and largely displace the lymphocytes in the follicles. No tumor metastases are found.

In a lymph node from the region of the left adrenal gland there is a large amount of fibrous tissue in the capsule, septums and about the lymph nodules which are filled with plasma cells and lymphocytes.

Polynuclear leukocytes are scattered among young blood cells about twice as large as lymphocytes with colorless or faintly acidophilic cytoplasm and with blue, granular, band-shaped nuclei.

In a tracheobronchial lymph node plasma cells and occasionally lymphocytes are widely disseminated so that follicles are not distinguished. There are a few mononucleated or multinucleated giant cells, and slight fibrous changes.

The stroma of the spleen is loosely arranged in fine and coarse bands which support compact masses of myeloid cells and occasional groups of lymphocytes so dispersed that the malpighian corpuscles are not distinguished. The metastatic nodules of tumor tissue are in all respects similar to those of other secondary growths.

A nodule from the diaphragm is composed of tumor tissue resembling that previously described.

The bony trabeculae of the left humerus takes a pale stain. The fat cells, sinuses, and all other normal structures of the marrow are replaced by dense interlacing bands of fibrous tissue with various hemopoietic cells (Fig. 4). Erythrocytes are few and megakaryocytes are numerous. These latter cells are elongated with maximum diameters of 40 to 60 microns and have from fifteen to eighteen compact, strongly basophilic nuclei surrounded by scanty cytoplasm. Other di- and trinucleated spherical cells with diameters as great as 40 microns are occasionally seen, as are lymphocytes, plasma cells and polynuclear leukocytes. Of the granulocytes "band" cells predominate. Neutrophilic myelocytes are abundant, while eosinophilic or basophilic are rare.

In a vertebra the moderately increased fibrous stroma supports hemopoietic cells similar to those noted in the left humerus. Megakaryocytes are infrequent while mature polynuclear leukocytes are numerous. In sections from one block, certain marrow tissues are necrotic.

The dense connective tissue framework which predominates in preparations of the left humerus is practically absent in ribs. A fine reticular network envelops evenly disseminated marrow cells more nearly uniform in size and shape than those described above. Of these cells, those of the polynuclear series are most abundant and megakaryocytes are few. Fat cells, not noted in other preparations of bone, are present.

Discussion

The clinical, hematological, and postmortem observations establish the diagnosis of chronic splenomyelogenous leukemia. The tumor of the left scapula is myeloblastic because it contains myeloblasts, myelocytes and megakaryocytes, and is malignant because of the metastatic growths in the spleen, liver, pleura and the axillary, biliary and cervical lymph nodes.

While sarcomas of the scapula are rare, no primary myeloid leukemia with sarcoma of this bone has been recorded. Fulghieri [5] described myeloid leukemia associated, in the later stages, with a myeloblastic tumor of the femur. In his report of a sarcoma of the skull with secondary growths in the spine, Ribbert [6] described cells like those of bone marrow, some of which contained hemoglobin. He considered them erythroblasts and was able to demonstrate transitional forms between erythrocytes and tumor cells. Christian,[7] in his report of multiple myeloma, stated that the description of erythroblastic cells in Ribbert's case was inadequate and that the hemoglobin content might be only stain from surrounding erythrocytes. Borst [8] mentioned malignant tumors with constituents like bone marrow in which occasionally he found all cells of the marrow but more commonly either megakaryocytes or leukocytes. Roman [9] studied the tissues from two patients who had died with bone sarcomas. In one, with multiple malignant tumors of the bone marrow, there was extensive invasion of the spinal canal and cranial cavity and metastases into the retroperitoneal, inguinal, popliteal and mediastinal lymph nodes. Histologically he found lymphocytes, plasma cells, granulocytes, and megakaryocytes in the tissues. In the second tumor, a sarcoma of the skull, spinal column and long bones, there were widespread lymph node metastases. Microscopically myeloid cells were identified. He referred to the reports of Marchand, Gussenbauer-Chiari, Dittrich and Schmidt which, in some respects, were similar to those he described. Fischer,[10] in his study of an endothelioma of the liver, observed cells like those of bone marrow, namely erythroblasts and myelocytes. In his differentiation between myeloid sarcoma (myeloma) and malignant giant cell sarcoma of bone Stewart [11] mentioned the rarity of islets of bone in the latter tumor and stressed the malignancy of the growth, in contradistinction to myeloma which he considered benign. Froboese [12]

likewise stated that myelomas are not malignant because metastases rarely, if ever, occur. This contention of Froboese was made after a study of a malignant growth of the sternum which contained myeloblasts, myelocytes, erythroblasts and erythrocytes with their intermediate forms. Although Wallgren [13] found many myelomas with secondary growths he considered myeloma benign. Mieremet [14] found megakaryocytes, erythroblasts, erythrocytes and various forms of polynuclear leukocytes in a tumor of the adrenal. Herscher and Thevenard [15] observed malignant myeloblastic sarcomas: two of the skull and one of the sternum. Megakaryocytes and myelocytes were noted in the tissues. Saleeby [16] reported two symmetrical tumor masses of hyperplastic bone marrow attached to the inner surfaces of the fifth ribs in a hemiplegic patient dying of bronchopneumonia. He briefly summarized the literature on heterotopia of bone marrow and recorded five cases not associated with either leukemia or pseudoleukemia. Warren [17] described an extensive retroperitoneal tumor simulating bone marrow and concluded that the hemacytoblast of Maximow was the predominant cell. In addition to the type cell, he found erythroblasts, erythrocytes, megakaryocytes and cells of the polymorphonuclear series. Hirsch [18] reported a myeloblastic sarcoma of the cranium with extensive local destruction and metastases to the cervical lymph nodes and right lung. He found myelocytes, leukocytes and megakaryocytes in the growth.

Summary

Chronic splenomyelogenous leukemia complicated by a malignant tumor of the scapula, with metastases to the axillary, biliary and cervical lymph nodes, liver, pleura, spleen and twelfth thoracic vertebra, is described in this report. Both the primary and secondary tumors contained cells resembling components of the bone marrow.

I wish to express my appreciation to Mrs. Eleanor A. Hunt of the Department of Anatomy of the University of Alabama for the illustrations in Figure 3.

REFERENCES

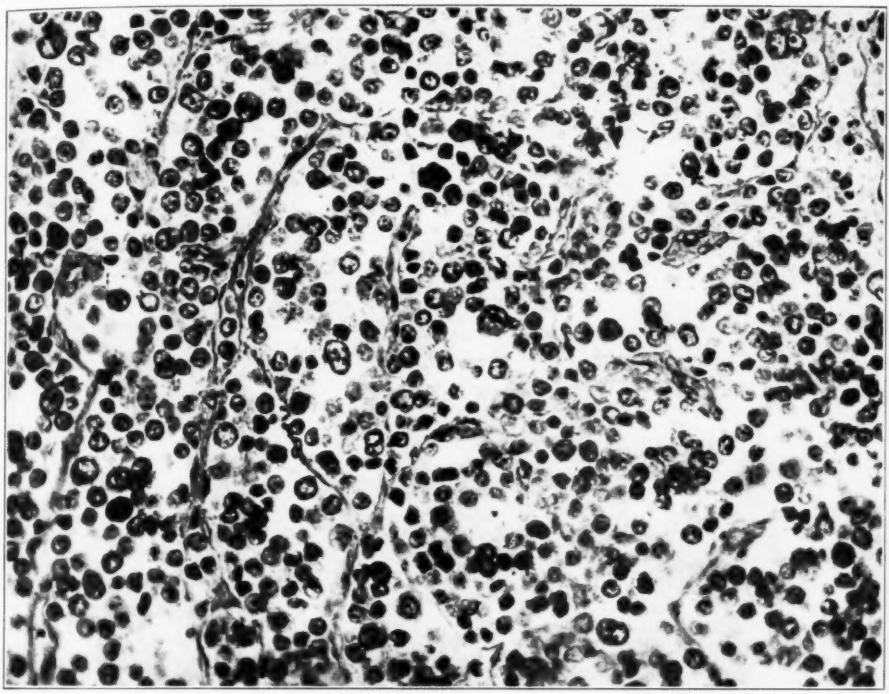
1. Mallory, F. B., and Wright, J. H. Pathological Technique. W. B. Saunders Co., Philadelphia, 1924, Ed. 8, 72, 149.
2. McClung, C. E. Handbook of Microscopical Technique. Paul B. Hoeber, Inc., N. Y., 1929, 252.
3. Romeis, B. Taschenbuch der microskopischen Technik. R. Oldenbourg, München and Berlin, 1928, Ed. 12, 367.
4. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1930, 117.
5. Fulghieri, U. Leucemia mieloide cronica a manifestazioni tumorali mieloblastiche. *Gazz. d. osp.*, 1927, **48**, 316.
6. Ribbert, H. Ueber das Myelom. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1904, **15**, 337.
7. Christian, H. A. Multiple myeloma. A histological comparison of six cases. *J. Exper. Med.*, 1907, **9**, 325.
8. Borst, M. Pathologische Anatomie, Aschoff, L. Gustav Fischer, Jena, 1913, Ed. 3, 700.
9. Roman, B. Zur Kenntnis der primären Tumoren des Knochenmarks. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1912, **52**, 385.
10. Fischer, B. Über ein primäres Angioendotheliom der Leber. *Frankfurt. Ztschr. f. Path.*, 1913, **12**, 399.
11. Stewart, M. J. Observations on myeloid sarcoma with an analysis of 50 cases. *Lancet*, 1914, **2**, 1236.
12. Froboese, C. Ein neuer Fall von multiplem Myelom (Erythroblastom) mit Kalkmetastasen in Lungen, Nieren und der Uterusschleimhaut. *Virchows Arch. f. path. Anat.*, 1916, **222**, 291.
13. Wallgren, A. Untersuchungen über die Myelomkrankheit. *Upsala läkaref. förh.*, 1920, **25**, 113.
14. Mieremet, C. W. G. Ein aus den verschiedenen Elementen des Knochenmarks bestehender Tumor in der Nebenniere. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1919, **30**, 403.
15. Herscher, M., and Thevenard, A. Un cas de myelome osseux multiple (sarcome myéloïde). *Ann. d'anat. path.*, 1924, **1**, 325.
16. Saleeby, E. R. Heterotopia of the bone marrow without apparent cause. *Am. J. Path.*, 1925, **1**, 69.
17. Warren, S. A malignant tumor simulating bone marrow. *Am. J. Path.*, 1928, **4**, 51.
18. Hirsch, E. F. Myeloblastic sarcoma of the cranium. *Am. J. Path.*, 1929, **5**, 303.

DESCRIPTION OF PLATES

PLATE 20

FIG. 1. Photomicrograph of the primary tumor to illustrate general configuration of the tissue. × 290.

FIG. 2. Photomicrograph to illustrate marked variation in size and shape of tumor cells in liver metastases. × 840.



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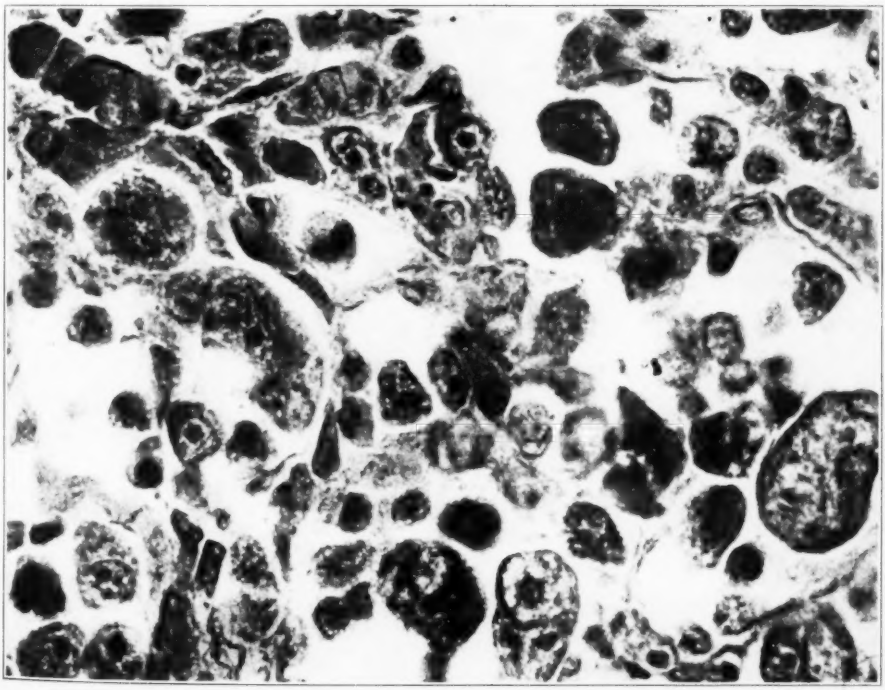


2

Dubois

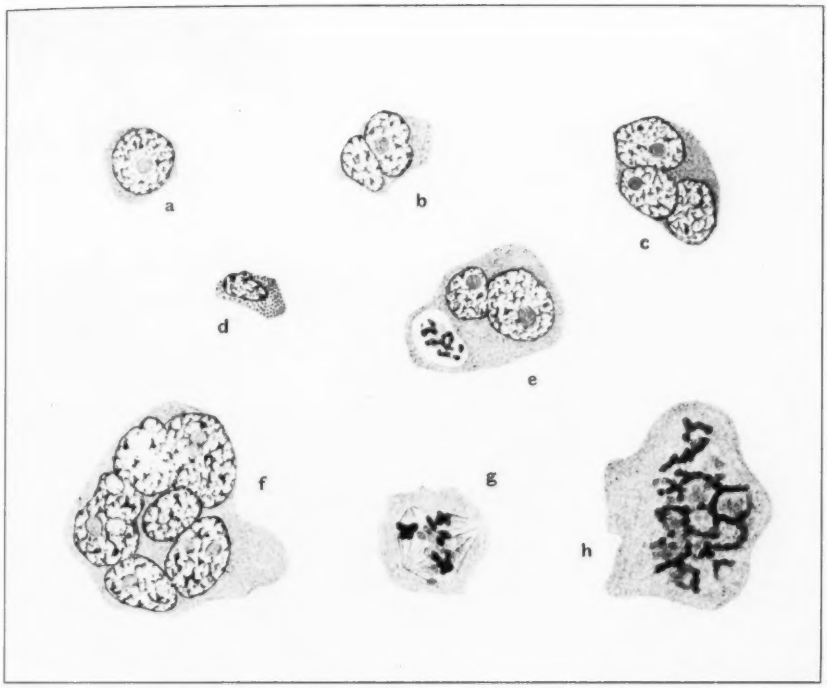
Myeloblastic Sarcoma of Scapula

PLATE 21

FIG. 3. Several types of tumor cells drawn at table level with a camera lucida. Approximately × 750.

a = Mononucleated tumor cell.
b = Binucleated tumor cell.
c = Trinucleated tumor cell.
d = Tumor cell with Ehrlich granules in cytoplasm.
e = Trinucleated tumor cells. Note one nucleus in mitosis.
f = Large multinucleated cell of the megakaryocytic variety.
g = Tumor cell in multipolar mitosis (metaphase).
h = Large megakaryocytic type of tumor cell in multipolar mitosis (prophase).

FIG. 4. Photomicrograph to illustrate interlacing bands of fibrous tissue and variety of hemopoietic cells in the marrow tissues of the left humerus. × 290.



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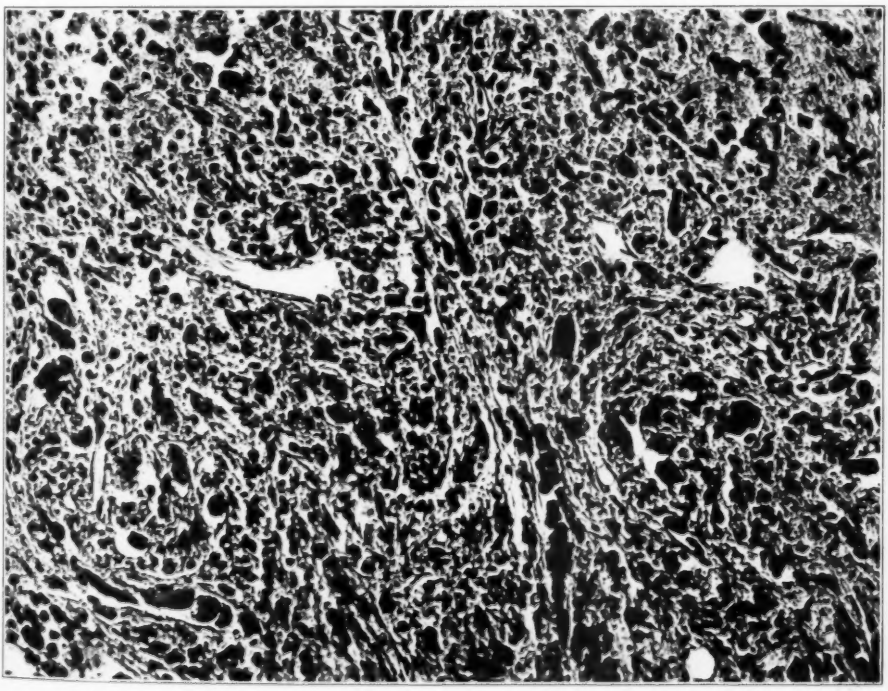

Dubois 4 Myeloblastic Sarcoma of Scapula

REPORT OF A CASE OF RETOTHELIOSARCOMA (RETICULOSARCOMA) OF THE CEREBRAL HEMISPHERE *

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As the tumor we are about to describe is of an almost unique character and presents considerable difficulties in the way of diagnosis, it seems desirable that the case should be reported in detail. Should one look through modern textbooks on brain tumors such as Cushing's [1] or Sachs' [2] one would find no description of an exactly similar neoplasm; nor have we been able to find any such detailed in shorter articles.

The specimen was removed at operation in two stages by Dr. B. N. Carter at the Cincinnati Children's Hospital, and submitted to our department for diagnosis. After a simple bone-flap decompression to relieve intracranial pressure, the tumor was later removed as completely as the condition of the patient allowed at a second operation. A third procedure, to extirpate what was left of the deeper extension of the growth, was not feasible. The tumor lay about 3 cm. beneath the cortex of the left temporoparietal region and extended deeply toward the central part of the brain, where it was infiltrating to such an extent that sharp dissection was necessitated. Otherwise, the tumor had a capsule which rendered enucleation relatively simple elsewhere. Dr. Carter reports that he explored the left ventricle and found its lining smooth and apparently unaffected by the growth.

REPORT OF CASE

Clinical History: The patient was a white girl, 9 years of age, who had been well up to about eight months ago. She was a normal, full-term delivered baby and, aside from having her tonsils removed a year prior to the onset of symptoms, had had no illnesses of consequence. The present attack began with severe frontal headaches, accompanied by vomiting of the projectile type, which came on about twice a week and were unaccompanied by nausea, usually following breakfast or supper. There was no abdominal pain. The child was bright and continued to go to school up to four weeks ago. She had had no disorders of

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taste, smell or hearing, but had a sudden attack of blindness following an X-ray examination about a month previous to admission to the hospital.

The essential findings on physical examination were as follows: The right pupil was larger than the left, the extraocular muscles acted normally, the optic discs were swollen and striated, their margins indistinct; the arteries of the fundi were contracted and their veins congested. There were no retinal hemorrhages. General physical examination was negative, save for the neurological findings. The knee jerks were absent and the Kernig, Babinski, Oppenheim and Romberg tests were negative.

During her stay in the hospital a right homonymous hemianopsia was determined and a diagnosis of left frontal tumor was made. After a month the Kernig sign became positive and the child's spleen was found to be palpable.

Following the operation there was paralysis in the right arm and leg, and ankle clonus on the right. The abdominal reflexes disappeared on that side also.

The patient died almost two months after the operation and, although every effort was made to obtain a permit for complete autopsy, permission was limited to an investigation of the brain. This was carried out by the hospital's pathologist, Dr. Irving H. Schroth, without any material addition to our knowledge of the case, as nothing was found except ventricular dilatation and foci of infection in the neighborhood of the operative site. It is greatly to be regretted that our case must, therefore, remain uncompleted.

The Tumor

Gross Examination: The specimen consisted of a mass 7.5 by 6.5 by 6 cm. in size, fairly well circumscribed and enclosed in a sort of capsule, excepting at one point where the surface was irregular and ragged. A few small tags of brain tissue adhered to the capsule. The section surface presented circumscribed, pearly white areas that alternated with serpigenous streaks or local spots of an orange-colored tissue that gave the tumor a xanthomatous appearance. The growth was rather nodular beneath its capsule and the pearly areas suggested cellular alveoli, while the orange material may have been necrotic tissue or a stroma with lipoid infiltration.

Microscopic Examination: Routine hematoxylin-eosin sections at once revealed that we were dealing with a most unusual brain tumor. Instead of the compact picture of a glioma, a meningioma, or that of an ordinary vascular or ependymal tumor, one finds that the greater part of the sections examined are composed of nests of enormous and very bizarre giant cells and syncytia which lie in a stroma of rather edematous connective tissue (Fig. 1). The cells have a

most pleomorphic appearance, ranging through the gamut of morphological variation — they may be spheroidal, turnip-shaped, fusiform, unipolar or bipolar with long tail-like processes, or they may be cylindroid (Fig. 2). They contain a varying number of nuclei, which may be single and of enormous size, or multiple, in which case there may be from ten to twenty or more nuclei of varying size and shape. Some cells are strewn with minute vesicular nuclei. Mitotic figures are very abundant and range from quasinormal karyokinetic forms to the most bizarre and abnormal, multiple, asymmetrical, cross- or y-shaped types. It would be impossible to describe a nucleus typical for this growth, so varied are these in their morphology; on the whole, however, they show a deeply stained but delicate chromatin network and one or more distinct and often enormous nucleoli. The cytoplasm of most of these cells is pale and vacuolated, though it may be slightly denser in the cell processes where it has become more compact. Micrometer measurements show that the large cells approximate 50 to 80 microns in their longest diameter, although one of them has attained a size of 192 by 80 microns. Their nuclei, when single, may be about 20 microns in long diameter, but one of the largest observed measured 40. The stroma of the tumor gives the general impression of trabeculae, between which are sinusoids that contain the tumor cells; one immediately thinks of the splenic architecture when one first looks at this tumor under low power (Roussy and Oberling[3]).

While casting about for the possible origin of such cells, it is natural to search for fields where they are most uniform in their morphology, most free from necrosis or degeneration and apparently in their most healthy condition, as evidenced by more normal mitotic figures. Such areas may be found in this tumor and they exhibit small, actively dividing polygonal cells which tend to be joined through anastomotic processes — cells that remind one of the retothelium (reticulo-endothelium) of the lymphoid or myeloid tissues. On the average, they measure about 8 microns in diameter and consist mostly of a somewhat vesicular nucleus and a narrow rim of cytoplasm with radiating processes.

As this tumor came from the brain, it might be derived from a number of sources: its healthier areas indicate a retothelial origin, but against this is the marked lack of vessels and the site of the growth; for although ordinary vascular tumors may be encountered

anywhere in the organ, the retothelial type is notably limited to the base, medulla, cerebellum and the spinal cord (Cushing,[1] Sachs,[2] Roussy and Oberling,[3] and Cushing and Bailey [4]). Other possible origins might be: (*a*) neuroglia, (*b*) ependyma, (*c*) meninges, (*d*) true nerve cells or ganglion cells. A metastatic origin cannot be excluded, but there is nothing in the history or physical examination to indicate another primary focus, unless it be the spleen or the tonsils. The spleen was not noted as enlarged until some time after admission. Concerning the tonsils we have no available pathological data, but they were not stated to be involved by neoplasia.

Special Methods of Examination

Neuroglia: We undertook impregnations of neuroglia cells and fibrils by means of a modified del Río-Hortega method on frozen sections (Foot,[5] and the new Wilder method on paraffin sections [6]); neuroglia fibrils were also sought with the familiar and reliable phosphotungstic acid hematoxylin stain of Mallory. These methods fail utterly to demonstrate any neuroglial elements whatsoever; the large cells do not impregnate black, as should derivatives of spongioblasts, but become either yellow or old gold. Their processes, it is true, are sometimes blackened, but not invariably, and they are never attached to vessel walls but trail off into the stroma.

Ependyma: The preceding methods should demonstrate ependymal cells as well as neuroglia, but they fail to do so. In the phosphotungstic acid hematoxylin sections one occasionally finds groups of tiny, violet granules clumped at the center of the cell, not far from its nucleus. Although this suggests the presence of blepharoplasts, it can scarcely be taken alone as an indication of ependymal origin in the face of the negative findings with neuroglial silver impregnations. Furthermore, the tumor does not obviously involve the wall of a ventricle.

Meninges: Meningeal origin, we think, may be ruled out on the basis of the site of the tumor and of its architecture, which in no way suggests that of a meningioma.

Neurocytes and Ganglion Cells: Here we encounter difficulties: sections impregnated by a modified silver nitrate method (Foot [7]) (Fig. 1), and by the Rogers method [8] show that the large cells may closely simulate ganglion cells; they have their general outline, their proc-

esses impregnate like the dendrites of pyramidal cells; and strange distorted forms resembling unipolar and bipolar neuroblasts may be found as gigantic cells. There are no demonstrable nerve fibers, however, and although one might construe some of the smaller cells that surround their larger fellows as being capsule, or satellite cells, this would require rather too much imagination, for such an arrangement is only occasionally found in this tumor. The cells lack Nissl bodies, which are well brought out by the silver nitrate method in normal nerve cells. The Rogers method, which picks out medullated and non-medullated fibers with precision, as a rule fails to demonstrate any here. The silver methods do impregnate the tails and processes of the fusiform and polar varieties of the tumor cells, sometimes giving them the appearance of coarse fibers, but this is also noted in the phosphotungstic acid hematoyxlin sections where they take on a deep blue stain occasionally.

Retothelial Cells: We are adopting the term "retothelial" for reticuloendothelial cells, as suggested by Roulet [9] in his paper on "Retothelsarkom." He says: "The term 'Retothelsarkom' is not only shorter than 'Reticuloendothelsarkom,' but it is likewise more correct, as the tumor takes its origin not from the *endo*thelium of the lymph sinus, but from the covering cells of the reticular framework, which are better termed '*reto*thelial' cells." This seems logical to us. Having considered the other possible origins of our tumor we are thrown back upon the first hypothesis of retothelial origin: let us test its validity in this case. Silver impregnation by the Foot and Foot method (Variant 3),[10] which is excellent for connective tissue, demonstrates the presence of abundant collagenous fibers in the trabecular, hyaline portion of the stroma; from this black reticulum fibers radiate into the cell nests. Where the tumor cells are large and atypical the reticulum seldom penetrates between them, although it may do so occasionally. The picture is somewhat like that seen in a carcinoma. Where the cells are smaller and more uniform in their morphology, in the areas already cited as probably constituting the most recent and "normal" tumor growth, the reticulum forms a rather dense network among the cells, running along their cytoplasmic processes and involving them in a loose felting of black fibers (Fig. 3). In these sections the resemblance to splenic tissue is much heightened by the presence of abundant collagen and some reticulum in the trabeculoid stroma.

Roussy and Oberling describe an "infiltration lipo-lipoidique" in their "angioréticulomes" and compare it to the infiltration seen in such dyscrasias as Pick-Niemann's disease, believing it to be a process expressing necrosis of brain tissue, setting free of lipoid substances from this and subsequent phagocytosis of this fatty material by the tumor cells. Frozen sections of our tumor, stained with scarlet R and hematoxylin, show a very rich lipoid content. Fine droplets form a sort of "milky way" in the hyaline portions of the stroma; there are collections of coarse drops and masses of this material at the periphery of necrotic areas and, most important, large numbers of droplets may be demonstrated in the tumor cells themselves (Fig. 4). These may be completely filled with lipoid material, so that they take on the familiar "mulberry" appearance — the cell body a vermilion mass, the blue nucleus embedded in closely packed red droplets which may be found in the cell processes — just as one sees melanin granules in those of melanoblasts. Other cells may show a few small lipoid droplets. A discussion of the lipoid content of the neuroglia may be found in a recent article by Sjövall.[11] The author cites Roussy and Oberling among other observers of this phenomenon. As the fat in the macroglia is deposited in smaller amounts than in the tumor cells under discussion and as Roussy and Oberling were cognizant of this when they mentioned it as a criterion of mesenchymal origin in the case of retothelial tumors, we may dismiss this as having but relatively slight importance in this case.

Discussion

With these data at our disposal we cannot seriously consider a neurogenic origin for this tumor, however alluring may be the apparent similarity of its larger cells to ganglion cells; all our evidence points more clearly to the retothelium. That these monstrous cells may be of retothelial origin is indicated by past experience (Foot [12]) as well as by comparison with more recent articles on the subject, such as Roulet's. Against this diagnosis stands the dictum that such tumors occur in the base, hind brain or cord, rather than in the hemispheres; there seems to be no valid reason why they should not be found there as well; it is probable that they merely do not happen to have been met with in that situation. This tumor differs from the cerebral retotheliomas hitherto described in its very active growth

and definitely sarcomatous nature. We have recently seen and studied one of the type described by Cushing and Bailey and by Roussy and Oberlin, which was located in the pontine region and corresponded exactly with their descriptions. In the case of that tumor there are abundant blood vessels and channels, but the mitoses and the very marked and bizarre distortions of morphology noted in our case are both absent. There are, it is true, numerous syncytial giant cells, but these are of a more orderly type of growth and exhibit few of the vagaries of those in the tumor we are discussing.

Authorities agree that these neoplasms originate in perivascular or intervascular congeries of retothelial cells, and the fact that they may be relatively poorly supplied with vessels is also recognized. It would appear that our case represents a malignant metamorphosis of such a tumor — Cushing and Bailey's "hemangioblastoma," or Roussy and Oberling's "angioréticulome." It would, under this hypothesis, constitute a retotheliosarcoma, or reticulosarcoma under the old terminology. One should avoid the use of the term "endothelioma" in speaking of such a tumor in order to escape the confusion that might arise between it and an "endothelioma of the dura." Many of the tumors described by Roulet as occurring in lymphoid tissue are so strikingly similar to ours in their appearance, that his Figs. 1, 2, 5, 6, 10 and 11 might have been taken from fields in our sections. There is no record of lymph node enlargement anywhere in our case and the spleen has only recently become palpable, which might be explained on the basis of a simple toxic splenitis. The chance of the tumor's arising in that organ seems to be remote, in view of the history and of the large size of the brain tumor before splenic enlargement was apparent.

Summary

A large tumor is reported, occurring in the left cerebral hemisphere of a 9 year old girl. The growth is described in detail and seems to have originated in the perivascular and intervascular retothelium. It differs from tumors hitherto described in its site, its manifestly malignant nature and more marked pleomorphism. Such tumors usually occur in the base of the brain, the hind brain, or the spinal cord; this one was deeply embedded in the left temporoparietal region. It shows extremely abundant mitoses and equally

marked variation in the size, shape and nature of its cells. It contains an abundant reticulum and its cells show marked lipoid infiltration of the "xanthomatous type." We believe that we have ruled out nervous or neuroglial origin by means of specific methods of impregnation and staining.

There is no evidence of a primary tumor elsewhere in the body, as determined by physical examination, and the symptoms in the case have been exclusively referable to intracranial pressure, no other symptoms having been observed in an otherwise healthy and normal child.

REFERENCES

1. Cushing, H. Intracranial Tumours. C. C. Thomas, Springfield and Baltimore, 1932.
2. Sachs, E. The Diagnosis and Treatment of Brain Tumors. Mosby Co., St. Louis, 1931.
3. Roussy, G., and Oberling, C. Les tumeurs angiomateuses des centres nerveux. *Presse méd.*, 1930, **38**, 179.
4. Cushing, H., and Bailey, P. Tumors Arising From the Blood Vessels of the Brain. C. C. Thomas, Springfield and Baltimore, 1928.
5. Foot, N. C. Suggestions for staining tumors of spongioblastic origin. *Am. J. Path.*, 1929, **5**, 215.
6. Wilder, H. C. Silver impregnation of glia and nerve fibers in paraffin sections after formalin fixation. *Am. J. Path.*, 1932, **8**, 785.
7. Foot, N. C. Two simple methods for the silver impregnation of nerve fibers in paraffin sections of the central and peripheral nervous system. *Am. J. Path.*, 1932, **8**, 769.
8. Rogers, W. M. New silver methods for paraffin sections. *Anat. Record*, 1931, **49**, 81.
9. Roulet, F. Das primäre Retothelsarkom der Lymphknoten. *Virchows Arch. f. path. Anat.*, 1930, **277**, 15.
10. Foot, N. C., and Foot, E. B. A technique of silver impregnation for general laboratory purposes. *Am. J. Path.*, 1932, **8**, 245.
11. Sjövall, E. Differenzierung und Funktionen der Glia. *Acta path. et microbiol. Scandinav.*, 1932, Suppl. xi, 79.
12. Foot, N. C. Report on a case of malignant endothelioma with necropsy. *J. Med. Res.*, 1924, **44**, 417.

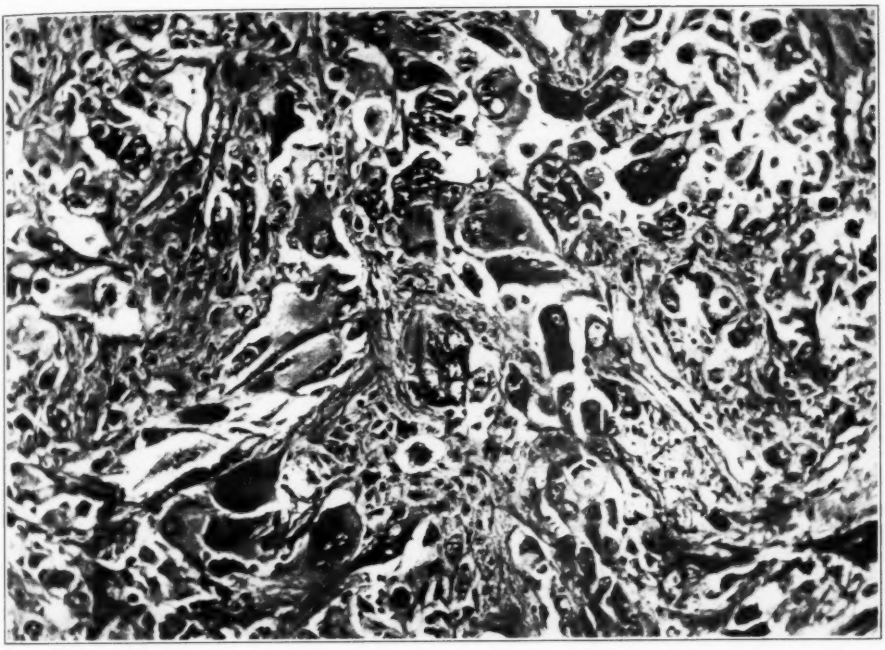
DESCRIPTION OF PLATES

The photomicrographs were all taken by Prof. J. B. Homan of our Department of Medical Art, with the assistance of the author.

PLATE 22

FIG. 1. Low power view of a field impregnated with silver nitrate. This shows the cell morphology and demonstrates the arrangement of the supporting tissue. × 200.

FIG. 2. High power view of a group of giant cells stained with phosphotungstic acid hematoxylin. Notice the clusters of small nuclei in some of the syncytia (8 microns in diameter). The large cell at the center, with a necrobiotic nucleus, measures 128 microns in length, the large nucleus just below it 32 microns and the darker nucleus below this 24 microns. × 800.



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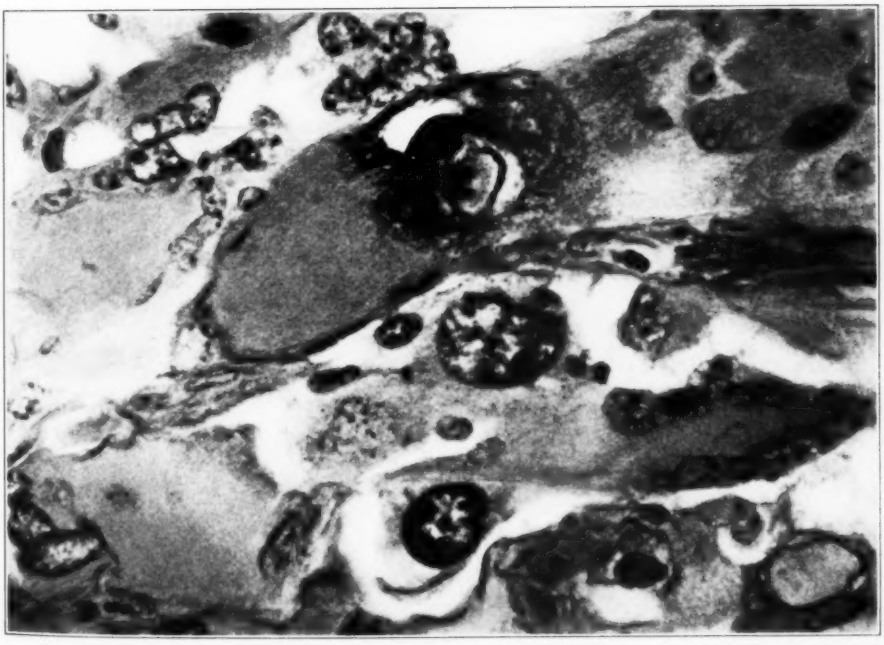


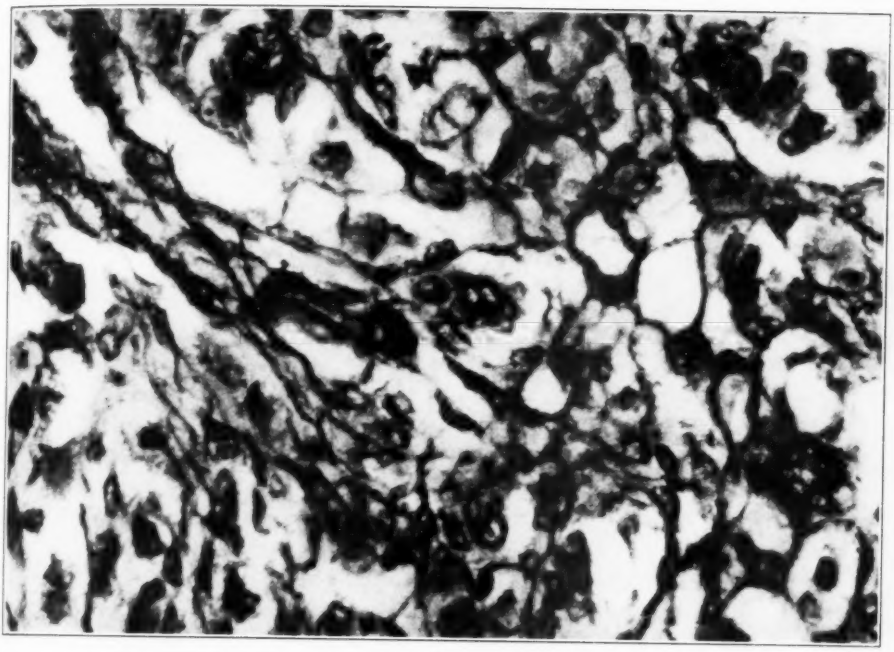
2

Foot Retotheliosarcoma of Cerebral Hemisphere

PLATE 23

FIG. 3. High power picture to show the reticulum in a section (Foot and Foot impregnation). One mitotic figure is present. The cells are more uniform in size and shape and resemble retothelial cells more closely than do the syncytia shown in the other figures. × 800.

FIG. 4. High power field from a frozen section stained with scarlet R and hematoxylin and photographed with a blue filter that makes the cellular detail insignificant while it emphasizes the lipoid droplets. The large cell, heavily laden with fatty material, measures 92 microns in its long diameter. × 800.



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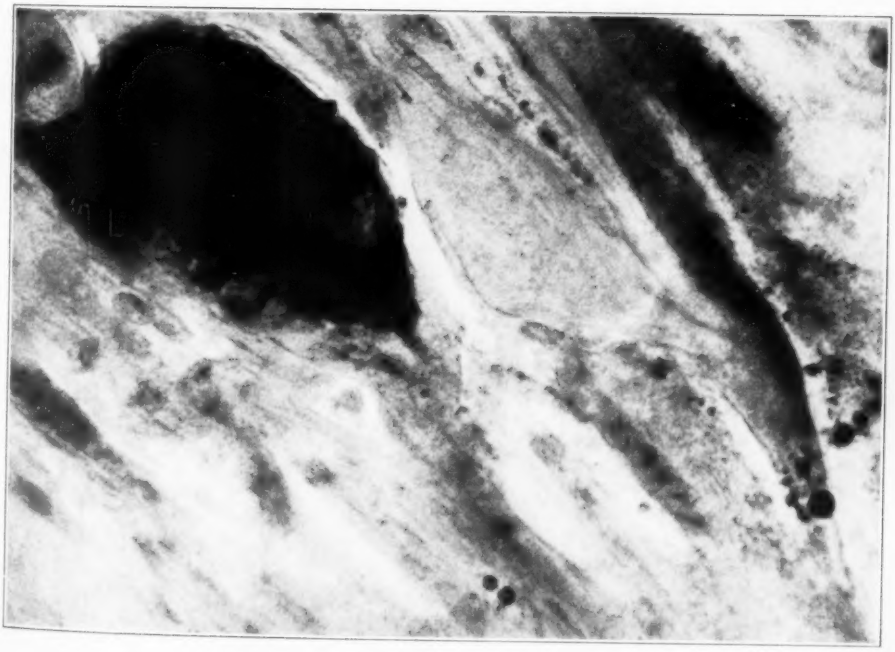

4

Foot

Retotheliosarcoma of Cerebral Hemisphere

EXPERIMENTAL STUDY OF THE EFFECTS OF POTASSIUM BICHROMATE ON THE MONKEY'S KIDNEY *

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Of the various metallic salts which have been most commonly employed as nephrotoxins experimentally, potassium bichromate stands as a rather poor third, being preceded by uranium nitrate and mercuric chloride. Undoubtedly there are good reasons for its lack of popularity but these are not often mentioned in the writings. From certain remarks in the literature one suspects that the chief objections to chromium compounds are the marked local irritative and corrosive properties and the violent gastro-intestinal manifestations. When administered subcutaneously compounds of chromium are quite likely to cause severe necrosis and sloughing at the point of inoculation, even though comparatively dilute solutions are employed. The same is of course true for corrosive sublimate. With both chromates and sublimate it is difficult to obtain constant results so far as the kidney is concerned because much of the poison may be held up at the point of entrance into the body in one animal, while in another quite the opposite may be the case. Ophüls [1] long ago recognized the irritative effect of potassium chromate and sought unsuccessfully to overcome it by mixing with the solution an equal amount of sodium carbonate. In a previous communication Ophüls [2] had observed the inconstancy of tubular necrosis in the kidneys of rabbits receiving quite large amounts of potassium bichromate.

The chromates have almost universally been regarded as pure tubular poisons, in contrast to uranium and mercury compounds which admittedly give certain evidences of damage to the glomeruli. Among the earlier descriptions of the renal lesions produced by chromic compounds that of Thorel [3] stands out because of his recognition, careful description and excellent illustration of the regenerative process in the affected tubules.

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Received for publication June 1, 1932.

There is rather general agreement in the literature that the chromates, like other metallic poisons, affect the proximal convoluted tubules predominantly or wholly. In his great work on the morphology of kidney secretion Suzuki [4] found that potassium bichromate damaged primarily the first and middle divisions of the proximal convoluted tubules, although if larger doses were administered the more distal part of the tubule was affected as well. Later Oliver [5] and still more recently Jessen [6] have confirmed Suzuki's observations in this respect.

As with uranium, practically all that is known about the renal manifestations of chromic compounds has been gained from experimental studies. In one of the earliest contributions in this field Kabierske [7] mentions the common employment of chromium as a tissue caustic in human beings, and instances of severe poisoning resulting therefrom. Major [8] has recorded a case of fatal chromic acid poisoning occurring in a man suffering from cancer of the cheek, treated after curettage with crystals of this substance. In view of the great rarity of such accidents it may be of interest to list some of the more salient clinical features of this case. The next day after application of the acid, the urine, which had previously been normal, contained 4 gm. of albumin per liter. Three days later the quantity had increased to 7.30 gm. per liter with hyaline and granular casts, red blood cells and numerous degenerated epithelial cells. After about forty-eight hours an almost complete anuria developed, but later the urine output was high and of low specific gravity. Death occurred on the twenty-ninth day, the blood urea at this time being 340 mg. and the creatinin 17 mg. per 100 cc. of blood. A definite acidosis developed but yielded readily to alkali therapy. At autopsy the kidneys presented a mottled appearance with the cortex swollen and the glomeruli very indistinct. There was no ascites or anasarca. Microscopic examination disclosed extensive destruction of the tubular epithelium with débris in the lumen and great swelling of the intact epithelial cells, especially in the convoluted tubules. Although a few mitotic figures were present in the cells of the affected tubules evidences of regeneration were not striking. For the most part the glomeruli appeared normal and in general the microscopic appearance was that of a pure tubular nephritis. Bernard [9] quite recently has described the clinical aspects of two instances of chromate poisoning in the human, one from his own practice, the other that of

Jonsson.[10] Both patients recovered after a stormy illness during which severe gastro-intestinal disturbances, characterized by vomiting and diarrhea, were as prominent a part of the clinical picture as the kidney disease. In each instance the poison had been taken by mouth.

MacNider [11] in a general review of acute experimental nephritis has well summarized the general opinion concerning the acute effects of chromate upon the kidney in the following words:

"1. The chromates induce a renal injury which is primarily tubular and affects in particular the epithelium of the convoluted tubules. Late in such a nephritis the glomeruli participate in the injury.

2. Secretory diuretics such as caffein, theocin, and theobromine sodium-salicylate are of no value and may be injurious.

3. The chromates lead to a rapid reduction in urine formation, an inability of the kidney to eliminate phenolsulphonephthalein, and a retention of chlorides, blood urea, non-protein nitrogen and creatinin.

4. The chromate nephritis is associated with the development of an acid intoxication which is likely a retention phenomenon and which is not favorably influenced by the use of an alkali."

Experimental Methods

Our interest in potassium bichromate as a nephrotoxin was aroused by the statements of Suzuki and others to the effect that it exhibits a selective affinity for a different division of the proximal convoluted tubule than uranium and sublimate, salts previously employed by Hunter [12, 13] in an investigation dealing with acquired resistance of regenerated tubular epithelium to metallic salts.

In view of Suzuki's statement that regenerated tubule cells after chromate poisoning were less resistant to subsequent and increased injections of the same poison we wished to determine if possible why this should occur with chromium compounds, whereas the opposite is true after injury by uranium and mercury. It appeared also that if potassium bichromate damages the end of the proximal convoluted tubule opposite to that injured by uranium such a localization would afford opportunity of observing the mode of epithelial regeneration and perhaps solve the problem as to the origin of the atypical flat-

tened cells, which in our experience were always found after poisoning with uranium and mercury.

Having available kidney material from normal monkeys and six living animals (*Macacus rhesus*) it was decided to utilize them for the problem after some rather disappointing preliminary experiments with rabbits. No attempt was made in either rabbits or monkeys to control the acidosis known to result from acute chromate poisoning, because we wished to produce as marked kidney damage as possible.

The essential data relating to dosage, duration of the experiments, clinical course and fate are summarized in Table I, but since the number of monkeys in the series is small it may be permissible to describe the findings in each case separately.

Monkey 1

Preliminary experiments with rabbits had shown that 1 cc. of a 2 per cent aqueous solution (0.02 gm.) per kilogram of body weight administered subcutaneously was near the lethal dose of potassium bichromate for this animal. Lacking available data as to the amount required to kill a monkey the first animal was given the same quantity per kilo as had been used for rabbits (36.3 cc. containing 0.726 gm. $K_2Cr_4O_7$). The rapidly fatal termination indicated that the dosage was far too great and that if subsequent experiments were to succeed the quantity must be decreased appreciably (Table I).

Macroscopically the kidneys displayed no changes.

Microscopic Examination: The epithelium of the entire proximal convoluted tubule stains faintly with eosin. Under high power the finer structure of the cytoplasm appears quite indistinct, the granules rarely being plainly outlined. The nuclei are unchanged. Only occasional cells show actual necrosis and desquamation. The lumina of many tubules contain a flocculent bluish substance but no distinct cast formation is noted in any of the tubules. The intertubular vessels of the cortex and medulla are almost uniformly engorged with blood. The glomerular capillaries, however, are frequently devoid of blood and it is rare to find more than two or three distended loops in any one glomerulus. The glomeruli for the most part do not fill the capsular space, which, aside from the presence of a few erythrocytes in a number of instances, contains no stainable material. In sections stained with hematoxylin and eosin it is difficult to distinguish

TABLE I

Potassium Bichromate Experiments with Monkeys

Monkey No.	Dates of injection	Duration of experiment	Site of injection	Amount	Clinical course	Fate
M–1	2/17/30	Less than one day	Subcutaneous	36.3 cc. 2 % Sol.	Injected 5 P.M. Found dead 7 A.M. on 2/18/30	Died
M–2	2/18/30	Less than one day	Subcutaneous	10 cc. 2 % Sol.	Injected 5 P.M. on 2/18/30 Found dead 7 A.M. on 2/19/30	Died
M–3	2/19/30 3/27/30 4/17/30 5/ 1/30	71 days	Subcutaneous " " "	2 cc. 2 % Sol. 2 cc. 2 % " 4 cc. 2 % " 6 cc. 2 % "	Nausea, vomiting and anorexia for one day after each injection	Died during the night of 5/1/30
M–4	6/30/30 7/21/30	22 days	Subcutaneous "	1 cc. 2 % Sol. 2 cc. 2 % "	Same as M–3	Died 12 hours after last injection
M–5	6/30/30 7/21/30 8/18/30 10/13/30 12/ 6/30	160 days	Subcutaneous " " " "	1 cc. 2 % Sol. 2 cc. 2 % " 4 cc. 2 % " 4 cc. 2 % " 5 cc. 2 % "	Same as M–3	Died about 20 hours later
M–6	6/30/30 7/21/30 8/18/30 10/13/30 12/ 6/30	163 days	Subcutaneous " " " "	1 cc. 2 % Sol. 2 cc. 2 % " 4 cc. 2 % " 4 cc. 2 % " 5 cc. 2 % "	Same as M–3	Killed 12/9/30

glomerular epithelial and endothelial cells because of the indistinctness of the basement membrane. The majority of the glomerular nuclei stain almost black with hematoxylin, indicating pyknosis. Even with the aid of the azocarmine stain it is not always possible to determine whether the pyknotic nuclei belong to epithelial or endothelial cells, but it appears that both are affected. The glomerular basement membrane stains faintly in comparison to that of the tubules. It does not appear to be split or beaded. Numerous orange-colored, droplet-like bodies of various sizes lie in the glomerular epithelial cells and capillary basement membrane.

Monkey 2

This animal received a single injection of 10 cc. of 2 per cent potassium bichromate (0.2 gm.); death occurred within 14 hours (Table I). The kidneys appeared normal grossly, but in microscopic sections there is evidence of damage to the epithelium of the entire proximal convoluted tubule, equally severe in all of the several divisions. The cell nuclei are condensed, small, deeply stained and not infrequently broken into granular bits, while the cytoplasm is swollen, filled with tiny vacuoles and narrows the lumen, in which there are frequently homogeneous hyalin-like eosinophilic masses varying in diameter but often about the size of an erythrocyte. The cells lining the other tubules are intact and unchanged with coarsely granular, acidophilic material present in their lumina, particularly the collecting ducts.

The glomeruli more nearly fill the capsular space than in Monkey 1, by reason of a greater blood content. The capsular spaces are empty. Azocarmine preparations reveal a diminution of both glomerular epithelium and endothelium, with a poorly stained, frayed and beaded capillary basement membrane. Droplets like those described in Monkey 1 are again numerous.

Monkey 3

Over a period of 71 days the monkey received four injections of potassium bichromate, totalling 14 cc. (0.28 gm.) Death came soon after the final administration of the drug. Further details of the experiment will be found in Table I.

The gross appearance of the kidneys was normal.

Microscopic Examination: Sections stained with hematoxylin and eosin disclose coagulative necrosis of the epithelium throughout the length of practically all proximal convoluted tubules. The degree of necrosis varies but generally is complete, often with a loss of individual cell outline. Nuclear pyknosis and karyorrhexis is evident everywhere in the involved tubules and all divisions of the proximal convoluted tubules appear to be equally affected. Much of the epithelium lining the other tubular units has desquamated, apparently as a result of postmortem changes. In the labyrinths the nuclei of the tubular cells stain unusually deeply with hematoxylin while the cytoplasm is distinctly acidophilic. About a few glomeruli are groups of relatively small and somewhat compressed tubules lined by flattened or low cuboidal epithelium with basophilic cytoplasm, representing segments of proximal convoluted tubules affected by earlier injections in which regeneration has taken place. Such tubules are encompassed by an abundance of connective tissue. The collecting tubules contain granular epithelial casts with an occasional pyknotic nucleus still present. Hyaline casts occur in certain of the collecting ducts. There is little blood in either the intertubular or glomerular vessels. The capsular spaces are large and empty. The majority of glomerular epithelial and endothelial nuclei are small and heavily stained. No apparent alterations are observed in the capillary basement membrane.

Monkey 4

As will be seen in the table this animal succumbed 12 hours following a dose of but 2 cc. (0.02 gm.) of potassium bichromate, after having recovered from the effects of a previous injection of 1 cc. of the same substance.

At autopsy the kidneys appeared normal.

Microscopic Examination: Sections reveal an intense swelling of the cells lining the entire proximal convoluted tubule, as evidenced by the increased granularity and pronounced acidophilic character of the cytoplasm. The nuclei show little change except beginning chromatolysis in some instances. Oftentimes the swelling is sufficient to close the lumina of affected tubules. In three places the origin of proximal convoluted tubules is made out and it can be demonstrated that the cells here are damaged in the same way and to the same ex-

tent as those farther along the tubule. Regenerative activity is not in evidence. None of the tubules contain casts.

The glomeruli are of average size and contain a moderate amount of blood. An increase of nuclei in the tufts and of the cells forming Bowman's membrane is present in all glomeruli with capillaries open sufficiently to permit a cell count. In addition the azocarmine stain demonstrates slight fraying and beading of the capillary basement membrane.

Monkey 5

Starting with an initial dose of 1 cc. of potassium bichromate the animal received altogether five injections (total 0.32 gm.), dying about 20 hours following the final administration and 160 days after the beginning of the experiment (see Table I for further details).

Macroscopically the kidneys are of average size, but on stripping the capsules there are seen on the cortical surface several small and shallow pit-like depressions.

Microscopic Examination: All blood vessels, including the glomerular capillaries, are fairly well filled. In the cortex there is evidence of very grave injury of the cells lining the convoluted tubules, distal as well as proximal. Although a good many such cells preserve their outlines well and retain their nuclei very few fail to show increased acidophilia of the cytoplasm and varying degrees of nuclear chromatolysis. The more frankly damaged cells exhibit coagulation of the cytoplasm and extreme condensation of the nuclei. Many are either in the process of desquamation or have already become loosened and lie free in the lumina. In a great many convoluted tubules cellular necrosis is complete with granular, pinkish detritus and fragments of nuclei lying inside the denuded and barren basement membrane, external to which are elongated, deeply staining nuclei of the interstitial connective tissue (Fig. 1). As yet there is no sign of regeneration of epithelium in the affected tubules, which is readily accounted for by the fact that the animal died less than one day following the last injection. Histologically it seems rather clear that only epithelium of the original and normal type has been affected by the nephrotoxin, since there are many tubules, which from their position in the labyrinths and proximity to glomeruli must be of the proximal and distal convoluted type, covered completely by an entirely different type of cell displaying no histological evidence of

injury. These tubules vary greatly in size and shape, are often small, irregular and tortuous, less frequently large and dilated. Always the lining epithelium is atypical for this location in that the cytoplasm is invariably basophilic and the cells are nearly all either flattened or low cuboidal. The nuclei are generally oval with the long diameter in the direction opposite that of the tubule, and contain a single large and prominent nucleolus. Individual cell borders are often obscure, suggesting a syncytium. Patency of the tubules is indicated by the presence of necrotic cells within their lumina. In places the new cells have grown over and enclosed bits of necrotic cellular débris staining brilliantly with eosin, in marked contrast to the bluish living cytoplasm about them. Rarely they are taller than normal, in palisade arrangement or heaped up in mounds. Calcification is lacking. Rarely a nucleus is observed in the process of mitotic division. In areas where entire groups of convoluted tubules have been relined by regenerated epithelium there is a distinct increase of interstitial tissue with slight lymphocytic infiltration. Henle's loop and the collecting ducts are filled with casts, mostly hyaline but sometimes vacuolated and apparently fatty.

The glomeruli fill the capsular spaces fairly well. Often the capsular epithelium seems to be increased but does not form epithelial crescents. In three instances erythrocytes are found in the capsular space. Under oil immersion a count of five glomeruli reveals 60, 59, 71, 56 and 73 capillary epithelial cells, a greater number than in any other animal of the series. The endothelial cells number 16, 16, 18, 16 and 18 in the same glomeruli. Their nuclei appear to be pyknotic. The basement membrane is slightly but definitely split and beaded. Coarse and fine hyaline droplets are numerous.

Monkey 6

The duration of this experiment was 163 days, the animal being killed 3 days after the final subcutaneous injection of 5 cc. of 2 per cent potassium bichromate (0.32 gm. total). In Table I will be found the amounts of the previous doses.

The gross appearance of the kidneys is quite like that described for Monkey 5.

Microscopic Examination: Microscopically there is evidence that the last dose of bichromate found little vulnerable epithelium on which to act, for nearly all convoluted tubules, both promixal and

distal, are found to be fully covered by cells of the regenerated type and only a comparatively few have been stripped bare of epithelium (Fig. 2). These show the usual picture of pyknosis and karyolysis of nuclei, coagulation of the cytoplasm and detachment from the membrana propria. The great majority of convoluted tubules are lined by atypical epithelium and are of all sizes and shapes, but tend to have large and dilated lumina almost invariably filled with granular, pinkish-staining débris. Oftentimes bits of such débris, and occasionally an entire necrotic cell with a shrunken nucleus, are found to have been overgrown by the proliferating new cells. Many of the regenerated cells are elongated, flattened and show little or no tendency to individuality. Frequently they are cuboidal and some even approach the normal morphology with spherical nuclei located near the base. Always, however, the cytoplasm is bluish. In some instances the basophilia is faint, but when such cells are examined under higher magnification it is found that the pinkish tinge is due to the presence of fragments of original epithelium enclosed by the new epithelium. Not infrequently regeneration has been excessive, the cells piling up in layers and partially or completely occluding the lumina. Mitoses are numerous and the majority of the nuclei are hyperchromatic (Fig. 3). There are numerous hyaline and fatty casts in the collecting ducts. Several sections include the glomerular end of proximal convoluted tubules and in all of these the lining cells are of the atypical flat or cubical regenerated variety. The interstitial tissue is slightly increased. The intertubular vessels contain a normal amount of blood. Although the capsular epithelium is increased in occasional glomeruli this is not striking or generalized. A few of the subcapsular spaces contain erythrocytes (Fig. 4). Capillary epithelial cells are more numerous than in the normal control kidney but are fewer than in the glomeruli of Monkey 5. Otherwise these cells are unchanged, but the endothelial nuclei stain deeply. The basement membrane of the glomerular capillaries is thickened and frayed. "Hyaline droplet" degeneration is well marked.

Discussion

From the foregoing descriptions it is quite evident that the kidneys of Monkeys 1 to 4 exhibit only acute lesions, Monkey 5 both acute and chronic damage, and that Monkey 6 is the only animal in which a truly chronic stage of renal disease was obtained.

The effect of potassium bichromate in the dosages employed is not restricted to the two segments of the proximal convoluted tubule nearest the glomerulus, but on the other hand is clearly diffuse throughout the length of the tubule. The effect upon the tubular cells is quite like that of uranium nitrate and mercuric chloride. Early there is swelling, vacuolization and increased acidophilia of the cytoplasm and either pyknosis, karyolysis or karyorrhexis of the nuclei. As necrosis becomes complete many cells lose their connection with the basement membrane, fall into the lumen, and with others form loose granular masses which become more compact and hyaline-like as the casts pass through the loop of Henle.

The irregularity of absorption of bichromate after subcutaneous injection is well illustrated by the difference in the renal lesions of Monkeys 5 and 6, receiving like quantities of poison over the same number of days. In the kidney of Monkey 5 the comparatively few tubular segments with regenerated cells indicates that up to the time of the final dose not a great deal of tubular epithelium had been destroyed. The animal died about twenty hours following the last injection. Previous experience with uranium and mercury had shown that regenerative activity cannot be expected before four to five days after administration of these substances and none was present in this case. On the other hand in the kidneys of Monkey 6 practically all of the original epithelium of both proximal and distal convoluted tubules must have been destroyed before the last dose was given, as evidenced by the presence of numerous new and uninjured cells and but few of the original type. All of the latter cells exhibit varying degrees of necrosis. It is significant that histological evidence of injury to regenerated epithelium is lacking, in spite of repeated injections of increasing size, while the original cells display frank and widespread necrosis. In this respect our observations do not coincide with those of Suzuki, who found a lack of resistance of newly formed tubular cells following bichromate poisoning. Our results indicate that such cells are resistant to potassium bichromate in much the same manner as Hunter [12, 13] had previously observed following the use of uranium and mercury.

Another interesting feature is the involvement of both proximal and distal convoluted tubules so strikingly apparent in Monkey 6. A careful study of the epithelial covering of these tubules reveals many cells in different stages of mitotic division, often already sur-

rounded by fully formed basophilic cells, but sometimes standing alone and stretching out to cover a partially persistent defect. The dividing nuclei belong to cells of the regenerated type, as shown by the strongly basophilic cytoplasm, a feature peculiar to renal epithelium formed after destruction of the original cells. From personal experience with uranium and mercury nephropathies we are able to say that cytoplasmic basophilia persists over a period of many months and is of greater value in distinguishing new from original cells than any other single criterion. Not infrequently the regenerated cells of bichromate and bichloride kidneys re-assume to a certain extent the normal cuboidal form, but the bluish coloration of the cytoplasm remains as a permanent marking. The importance of this feature seems rather generally to have been overlooked, although it was depicted clearly by Thorel in 1903 and again described by Oliver in 1915.

A further point of much interest is the morphology assumed by regenerated tubular epithelium. Long ago Suzuki noted the flattened character of tubular epithelium formed after uranium poisoning and advanced the opinion that it might represent an ingrowth of cells from the thin limb of Henle. Somewhat later Oliver made the same observation as to the morphology, but felt that flattening was due to hindrance by the connective tissue proliferation which developed conjointly with the epithelial changes. Oliver saw even in severe cases where the tunica propria was practically denuded a persistence of a few cells with normal nuclei but altered cytoplasm, from which he believed regeneration took place. An occasional mitotic figure was found in the slightly damaged first division of uranium kidneys, but there was even less proliferation of the cells lining the thin limb of Henle's loop. Later still Hunter [12] confirmed Oliver's observations. Quite recently MacNider [14] has asserted that in dogs poisoned with uranium nitrate more than one kind of repair may be instituted to replace the loss of cells in the proximal convoluted tubules. When the initial injury was not too severe, as measured functionally by the severity of response and rapidity of recovery, the type of repair was found to be affected by the formation of cells from preëxisting tubular epithelium normal for the location of the injury. A subsequent injection of uranium caused necrosis of this normal form of regenerated cell and indicated a lack of resistance or even in-

creased susceptibility if the dogs were pregnant. A second variety of repair consisted in a replacement of convoluted tubular cells by flattened atypical forms "originating as an ingrowth from below . . . less specialized in structure and apparently in function" (see page 429). The latter form MacNider recognized as resistant to uranium and felt that its development might constitute a part of the defense mechanism of the kidney. Although MacNider does not state the exact source of the flattened cells the inference is that the flattened cells come from the descending limb of Henle's loop. We are unable to subscribe to this view, but rather agree with Oliver that flattening is probably the result of connective tissue proliferation, which we have found not only after damage of the kidney by uranium, but also to a slight extent with potassium bichromate. Further, it is not unreasonable to assume that regenerating renal tubular epithelium may possess the faculty of elongation in order to fill in defects quickly and establish continuity in much the same manner as Hartwell [15] has shown takes place in the healing epidermal wounds. Still another factor tending to produce flattening is pressure exerted by casts within the lumina of affected tubules. Moreover, as we have described in one animal (Monkey 6), not only is the entire proximal convoluted tubule lined by cells of the atypical, and in part flattened, type but the distal convoluted tubule as well. If then, as is true in this instance, the ascending limb of Henle's loop has normal epithelium it would be quite impossible for an ingrowth of cells from below to take place. Certainly the new epithelium covering the distal convoluted tubules must have originated from preëxisting cells at this point, yet the morphology of the cells is identical with those in the proximal convoluted tubule.

Frequently the newgrowth of epithelium in bichromate nephropathy is excessive, the cells heaping up in mounds forming syncytial giant cells or being laid down in layers. Although the intent of such regenerative activity is good, harm may result from narrowing or occlusion of the lumen.

A point of great similarity between the renal picture of bichromate and mercuric nephropathies is the failure of necrotic cells to be swept out of the tubules promptly. Often remnants remain at the site of injury for some time after regeneration has begun and are bridged over by new cells, giving the impression of phagocytosis.

The inclusions are readily recognized by their strong eosinophilic staining, contrasting sharply with the bluish cytoplasm of the regenerated epithelium.

Potassium bichromate, like uranium nitrate and mercuric chloride, produces glomerular changes which are demonstrable with the aid of the azocarmine stain. A detailed description of these alterations will be found in another paper.[16] In this communication it is sufficient to state that in acutely damaged kidneys there is a diminution in the number of glomerular epithelial and endothelial cells, hyaline droplet degeneration, and sometimes fraying and beading of the capillary basement membrane. The chronic stages are marked by an increase of epithelial cells, thickening, fraying and splitting of the basement membrane. In the small series of monkeys studied the glomerular lesions are less constant and less severe than those in rabbits receiving uranium nitrate and mercuric chloride.

Summary

1. Potassium bichromate in dosages employed in the foregoing experiments with monkeys affects diffusely the proximal and distal convoluted tubules and glomeruli of the kidney. The type of injury is quite comparable to that induced by uranium nitrate and mercuric chloride.

2. The drug is locally corrosive when administered subcutaneously and the quantity absorbed varies considerably in different animals of the same species. Definitely chronic renal lesions were obtained in but one animal out of six.

3. Following repeated injections of potassium bichromate there occurs a regeneration of tubular epithelium of distinctly atypical morphology which is resistant to further injury by the nephrotoxin.

REFERENCES

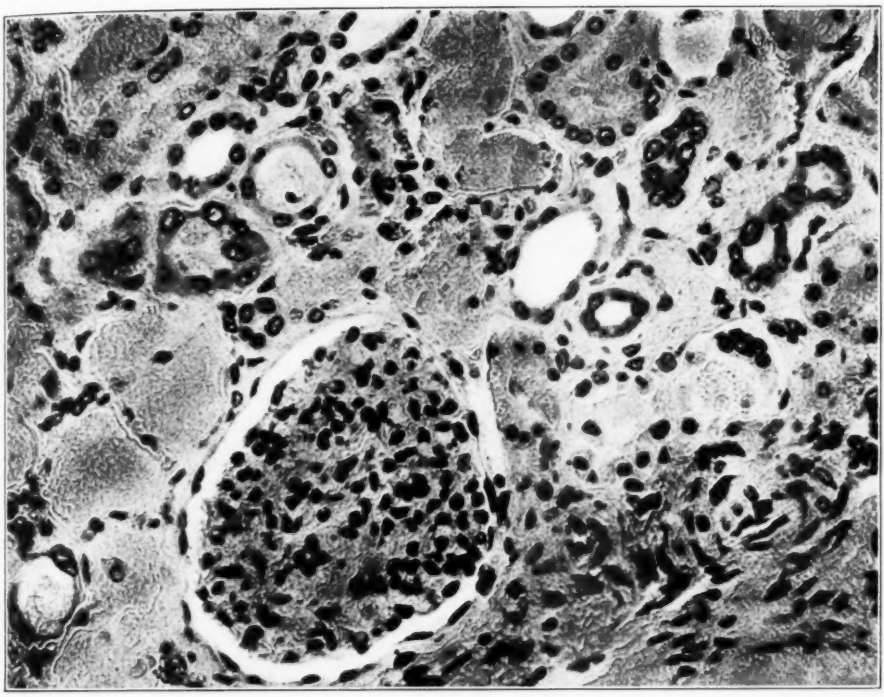
1. Ophüls, William. Experimental nephritis in guinea-pigs by subcutaneous injections of chromates. *Proc. Soc. Exper. Biol. & Med.*, 1911–12, **9**, 11.
2. Ophüls, William. Some interesting points in regard to experimental chronic nephritis. *J. Med. Res.*, 1908, **18**, 497.
3. Thorel, Ch. Pathologisch-anatomische Beobachtungen über Heilungsvorgänge bei Nephritis. Eine experimentelle und kritische Studie. *Deutsches. Arch. f. klin. Med.*, 1903, **77**, 29.
4. Suzuki, Tatzuo. Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen. G. Fischer, Jena, 1912.
5. Oliver, Jean. The histogenesis of chronic uranium nephritis with especial reference to epithelial regeneration. *J. Exper. Med.*, 1915, **21**, 425.
6. Jessen, J. New research on chromic nephritis. *Hospitalstid.*, 1925, **68**, 921.
7. Kabierske, Eugen. Die Chromniere. Inaug. Diss. Breslau, W. G. Karn, 1880.
8. Major, Ralph H. Studies on a case of chromic acid nephritis. *Bull. Johns Hopkins Hosp.*, 1922, **33**, 56.
9. Bernard, E. La néphrite chromique. *Prat. méd. franç.*, 1929, **8**, 466.
10. Jonsson, E. Ett fall av akut kalium bikromatförgiftning. *Hygiea*, 1927, **89**, 84–91. (Cited by Bernard [9].)
11. MacNider, Wm. de B. A review of acute experimental nephritis. *Physiol. Rev.*, 1924, **4**, 595.
12. Hunter, Warren C. Experimental study of acquired resistance of the rabbit's renal epithelium to uranyl nitrate. *Ann. Int. Med.*, 1928, **1**, 747.
13. Hunter, Warren C. Experimental study of acquired resistance of the rabbit's renal epithelium to mercuric chloride. *Ann. Int. Med.*, 1929, **2**, 796.
14. MacNider, Wm. de B. The functional and pathological response of the kidney in dogs subjected to a second subcutaneous injection of uranium nitrate. *J. Exper. Med.*, 1929, **49**, 411.
15. Hartwell, Shattuck W. Surgical wounds in human beings. A histologic study of healing with practical applications. I. Epithelial Healing. *Arch. Surg.*, 1929, **19**, 835.
16. Hunter, Warren C., and Roberts, Joe M. Glomerular changes in the kidneys of rabbits and monkeys induced by uranium nitrate, mercuric chloride and potassium bichromate. *Am. J. Path.*, 1933, **8**, 665.

DESCRIPTION OF PLATES

PLATE 24

FIG. 1. Photomicrograph of a representative area from the kidney of Monkey 5. Three segments of a convoluted tubule to the left of the glomerulus show complete epithelial necrosis, leaving only the barren membrana propria and nuclei of interstitial cells to mark the outlines of the tubules. Alongside these and near the corner is a segment of a tubule showing the hyperchromatic nucleus and scanty cytoplasm of a newly formed epithelial cell. To the right of the glomerulus are two convoluted tubules fully relined with atypical regenerated epithelium, and slightly below them is another containing a syncytial multinucleated giant cell. Three convoluted tubular segments in the upper right hand margin exhibit evidences of epithelial injury — nuclear pyknosis and karyolysis and swelling of the cytoplasm.

FIG. 2. Low power photomicrograph from the kidney of Monkey 6 showing a labyrinth with portions of a medullary ray on either side. Few, if any, proximal or distal convoluted tubules retain the original form of epithelium, but instead are lined by flattened or irregularly shaped cells. Certain of the tubules are dilated and contain hyaline casts, others are filled with granular débris. Hematoxylin and eosin stain.



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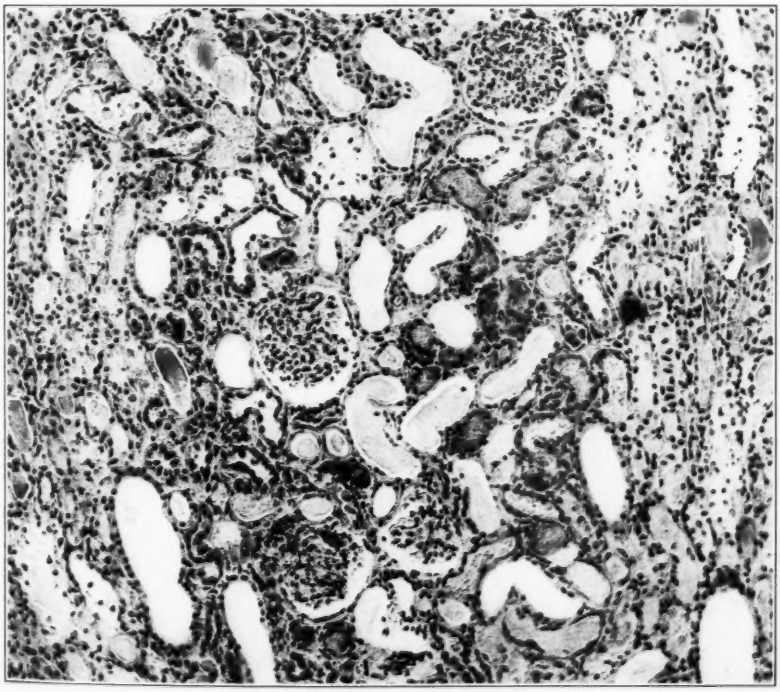

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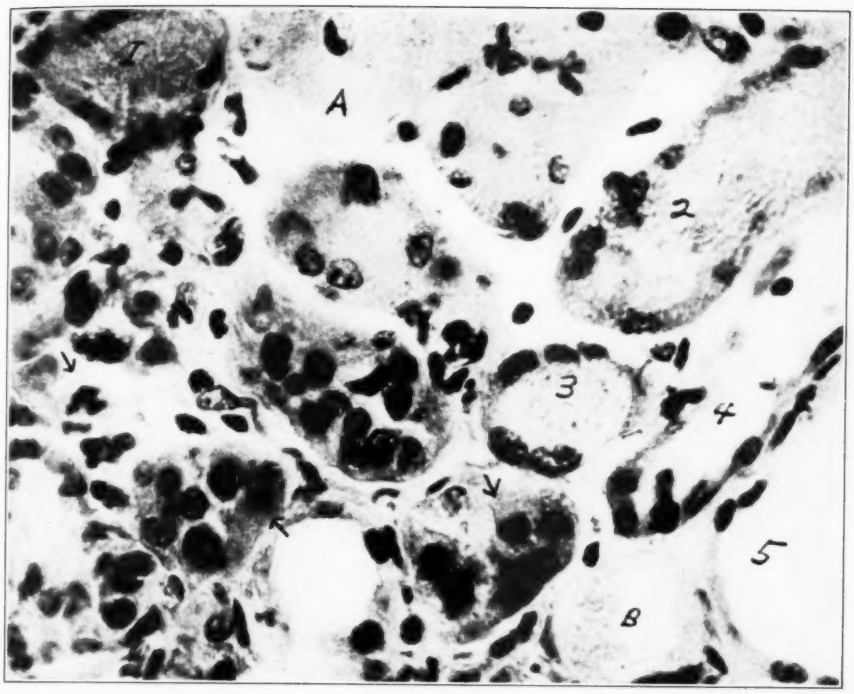
Hunter and Roberts

Effects of Potassium Bichromate on Kidney

PLATE 25

FIG. 3. Monkey 6. Photomicrograph from a section stained with hematoxylin and eosin. High power. The varied morphology of regenerated convoluted tubular epithelium is well exemplified in this field. The lining epithelium of the three tubules containing mitotic nuclei (indicated by arrows) is distinctly irregular and almost occludes the lumina. In Tubules 1, 2, 3, 4 and 5 the regenerated cells are quite flat and their basophilic cytoplasm stains deeply. A and B are tubules in which complete necrosis has occurred. Note the lack of evidence of injury to regenerated epithelium.

FIG. 4. Another field from the same section as Fig. 3. Note erythrocytes and albumin in subcapsular space, pyknosis of glomerular endothelial and epithelial nuclei and slight increase of interstitial tissue external to glomerulus. On the left are three convoluted tubules almost occluded by an overgrowth of atypical regenerated epithelium. The arrows point toward a fragment of a necrotic cell over which new epithelium with dark basophilic cytoplasm is beginning to grow.



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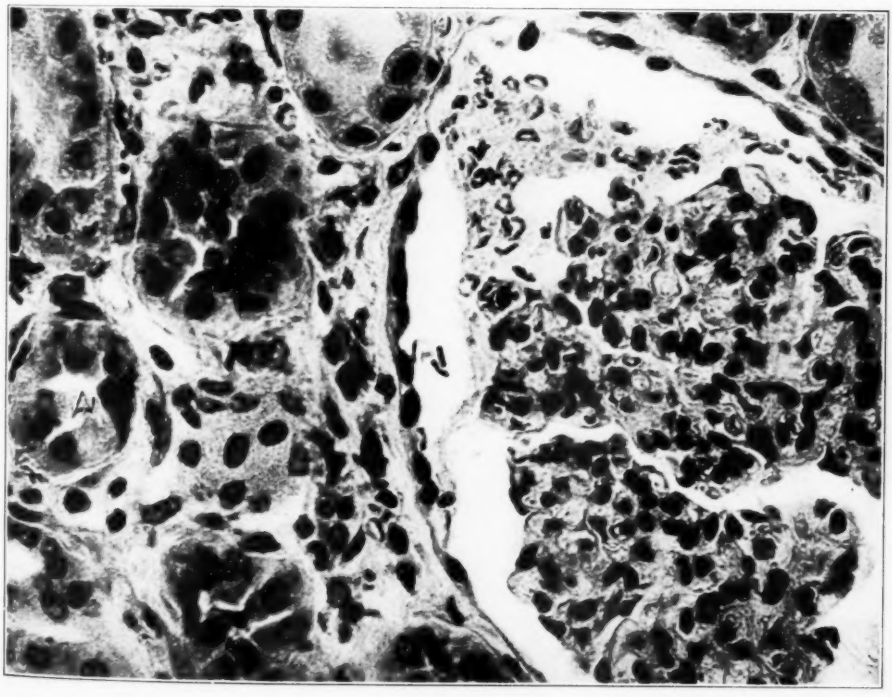

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Hunter and Roberts

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Correction

In Volume IX, No. 1, on page 116 the two statements beginning in the third line of the third paragraph should read as follows: "The narrow margins of cytoplasm contain oxydase granules. Other cells similar, but with no granules, resemble the hemocytoblasts of Maximow."